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Investigating Salt Stress Resilience in *Brassica oleracea*



**A thesis submitted for the Degree of
Doctor of Philosophy**

By

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List of Abbreviations

The following abbreviations are used in this Thesis write-up

ABA	abscisic acid
ABRE	abscisic acid-response element
AC	Anther culture
ATP	adenosine triphosphate
cAMP	cyclic-adenosine monophosphate
CAX	cation/H ⁺ exchanger family/genes
CBF	cold-binding factor
CDPK	calcium-dependent protein kinase
cGMP	cyclic-guanosine monophosphate
VCLC	Vacuolar gated chloride channels
CNGCs	cyclic nucleotide-gated channels
COR	cold-regulated
CRD	completely randomised design
CV	coefficient of variation
DH	doubled haploid
DMSO	dimethyl sulfoxide
DNA	deoxyribose nucleic acid
DRE	dehydration-response element
DREB	dehydration-response element binding protein
ELS	embryo-like structure
FDR	false discovery rate
FAO	food and agricultural organization

GLRs	glutamate receptors
HAK	high-affinity K ⁺ uptake transporter
HKT	high affinity potassium transporter family
ICP-MS	inductively coupled plasma mass spectroscopy
IMC	isolated microspore culture
KT	potassium transporter
MAP	mitogen activated protein
MAS	marker-assisted selection
mRNA	messenger RNA
NHX	Na ⁺ /H ⁺ antiporter family
NSCCs	non-selective cation channels
qPCR	quantitative real time polymerase chain reaction
QTL	quantitative trait locus/loci
r	correlation coefficient
RD	responsive to desiccation
RNA	ribose nucleic acid
ROS	reactive oxygen species
RT-PCR	reverse transcriptase polymerase chain reaction
SI	self-incompatible/self-incompatibility
SKOR	stellar K ⁺ outward rectifying channel
SNPs	single nucleotide polymorphisms
SOS1	salt overly sensitive pathway/genes
V-type	vacuolar type
TFs	Transcription factors
DFS	Diversity foundation set

DFFS	Diversity fixed foundation set
SOS	salt overly sensitive
SAGE	serial analysis of gene expression
KORCs	K ⁺ outward rectifying channels
VIC	voltage-independent cation channels
NSCCs	non-selective cation channels
KUP	potassium uptake gene
SKOR	stellar K ⁺ outward rectifying channels
24 hrpt	twenty-four hours post first salt-treatment
2 wkpt	two-weeks post first salt-treatment
8 wk 24hrpt	eight-week 24 hours post second salt-treatment
10 wkpt	two-weeks post second salt treatment

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Declaration

I hereby declare that the work contained in this thesis is the original work of the author, except where specific reference is made to other sources, with the nature and extent of the author's contribution indicated (as appropriate) where work was based on collaborative research. The work was undertaken at the School of Life Sciences, University of Warwick between September 2013 and July 2018 and has not been submitted, in whole or in part, for any degree, diploma or other qualification.

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Abstract

Soil salinity remains a global problem that affects approximately 20% of irrigated land and reduces plants growth and crop yields. Many of the *Brassica oleracea* species are important horticultural crops. The work aims to study variation in *B. oleracea* genotypes in response to salt stress and involves the use of cultivated *B. oleracea* DHSL150, wild *B. oleracea* lines and doubled haploid (DH) lines derived from them. Plants were exposed to salt stress through salt-shock using 250 mM NaCl at week-six of their growth. Morphological traits were measured including; plant height, plant fresh/dry weights, leaf fresh/dry weights and leaf area. Na⁺, K⁺ and Ca²⁺ were measured by using inductively coupled plasma mass spectrometry (ICP-MS) from leaf mineral. The results indicate significant variation between the lines, for example growth reduction of -11.74% to -14.96% was observed in the parent lines and, -1.84% to -11.24% in the DH lines compared against the untreated control. Plant fresh and dry weights exhibited a reduction between -32.92% to -2.15% in the wild S1parent lines and -20.14% in the parent DHSL150 line while their DH lines reduced by -28.52% to -12.42 %. Leaf morphology affects the leaf area, which showed a significant reduction in DHSL150 and four other wild S1 parent lines (p <0.05) as compared to the untreated control. The leaf area of some DH lines showed no difference compared to the control, however others exhibited a significant reduction (p <0.05). Analysis of mineral content within leaves revealed significant variation between the *B. oleracea* genotypes 24 hr post-exposure to salt treatment. The leaf Na⁺ level showed a significant increase in all the genotypes while K⁺ and Ca²⁺ level showed a non-significant reduction 24 hr post-treatment. Two-weeks post-treatment, K⁺ level were affected. It dropped significantly in some lines especially in DHSL150 thereby affecting K⁺/Na⁺ ratio. A significant K⁺/Na⁺

ratio increase was observed in some wild S1 accessions and DH lines, which indicates resilience to salt stress. A successful strategy for salt tolerance relies on the maintenance of a high K^+/Na^+ ratio through a mediated active/passive transport systems carried out by ion membrane transporters/channels. The relative gene expression of some selected ion membrane transporters using qPCR was carried out. Relative gene expression of Na^+/H^+ antiporter (*NHX1*), and potassium transporters (*KT9* & *KUP11*) increased between treated and the control with *B. oleracea* wild S1 and DH lines both showing improved K^+/Na^+ two-weeks post-treatment. Other transporters such as vacuolar H^+ adenosine triphosphatase (*V-ATPase -G*), and chloride gated channel (*V-CLC*) also showed increased expression with respect to the untreated control. Variation due to the effects of allelic variants was investigated using genotype-by-sequencing (GBS). A significant variation at different chromosome positions corresponding to the introgressed regions in the DH lines was observed between parent lines. These finding could be significant for plant breeding and development of enhanced varieties of brassica vegetables with salt resilience.

CHAPTER ONE

General Introduction

1.0 Introduction

Under the United Nations programme on Sustainable Development Goals (UNSDGs, 2017) presented an urgent and formidable challenge to scientists and society alike, citing the need for agricultural transformation and food sector in order to achieve food and nutrition security, others include ecosystem sustainability, economic growth, and social equity over the coming decades. Global food demand has been predicted to grow by 70 - 85% as world population suggested to possibly reach 9.15 billion by 2050 (www.fao.org/economic/esa) (FAO, 2017). These problems are predicted to be exacerbated by the effect of climate change and thus have far-reaching implications for global food security. The predicted increase in drought and intense precipitation, elevated temperatures, as well as increased salt and heavy metals contamination of soils and the effects of pest and pathogen infestations would certainly take a major toll on crop yields (Long et al., 2015). Different environmental stresses are among the main causes of a decline in crop productivity worldwide leading to billions of dollars losses (UNSDGs, 2017). For example, the suggested agricultural losses related to high soil salt are of serious concern both in food production and for the substantial farmable area affected, where 50% of total land mass will be lost due to salinity (Ilanguvaran and Smith, 2017). The salt-affected lands identified are often in arid and semi-arid regions and this could have a direct conflict with the burgeoning world population posing a significant challenge for sustainable food supplies (Kumar, 2013). Also, the problem of salt stress conditions has been exacerbated by unhealthy human activities, such as illegal mining, explorations, dumping of hazardous substances in oceans and waterways and improper irrigation practices championed by lack of artificial or natural drainage systems (Kumar, 2013). Such practices expose our fertile agricultural land to unwanted and deleterious salts substances. One of the worst being improper irrigation without drainage management which is a source of salt, which can accumulate in the root zone of the plants,

thereby affecting the soil properties and crop productivity (Ram et al., 2008). An FAO report has shown that over 830 million hectares of arable land worldwide are affected, by either saline water (403 million hectares) or by the conditions related to soil sodicity (434 million hectares; FAO, 2008). Additionally, more than 6.0% of the entire land of the world is shown to be involved and the agricultural cost implication is worth of billions of US dollars annually and expected to increase due to the effect of global warming (Metternicht and Zinck, 2003; Yensen, 2008; Patel et al., 2011).

1.1 Health and Economic Benefits of *B. oleracea* Vegetables

The *B. oleracea* family, include; the broccoli, cabbages, kale, cauliflower, and Brussels sprouts, which are essential vegetables for nutrients excellent sources of vitamins, minerals and fibre good for the treatment of diseases; such as incidence of type II diabetes and coronary heart disease (Walley and Buchannan-Wollaston, 2011; Ortega et al., 2012). They could also be used to improve receptor sensitivity to insulin (Harris, 2008). The *B. oleracea* vegetables contain a high level of minerals that are essential components of healthy living. Deficiencies of such minerals could lead to conditions such as anaemia (iron), rickets (calcium and vitamin D), influenza (selenium), reduced immune function, and cognitive decline (zinc) (Beck et al., 2001; Rayman, 2012; Chasapis et al., 2012).

The economic value of individual of *B. oleracea* crops has been reported to contribute significantly in many countries of the world. For example, in 2004, the United Nations Agriculture Organisation (FAO) database reported that over three million hectares (ha) and eight hundred thousand ha used for cabbages and cauliflowers respectively. Production has shown to amount of over sixty-eight million and sixteen million metric tons (Mt) produced for cabbages and cauliflower. The amount has shown to increase in 2010 where over 76

million tons of *Brassica* vegetables have been produced worth over 14.85 billion US dollars (<http://faostat.fao.org/>). Record in 2017 shows that the producers have shown to increased worldwide from the FAOSTAT database over 105,494,481 million tons of cabbages and related *B. oleracea* crops have been produced (<http://faostat.fao.org/>).

1.2 Taxonomy of *Brassica oleracea* species

The taxonomy of *Brassica oleracea* (*B. oleracea*) species shows that it comprises a number of important vegetative crops such as cultivars, including; cabbage, cauliflower, broccoli, Brussels sprouts, kohlrabi, kale (**Figure 1.0**). The cultivated *B. oleracea* crops have shown to derived their common origin from the wild forms of the *B. oleracea* species. The *B. oleracea* species shows a clear morphological diversity and crop forms. Various members are grown for their leaves, flowers, and stems, with different parts of the plant being consumed as vegetables (Liu et al., 2014). Most of the varieties of *B. oleracea* crops have been in cultivation for time immemorial that little details remain of how they are originated (Gray, 1982). The uncultivated forms of *B. oleracea* have been the wild cabbage and usually found around the coastal regions especially south and western Europes (Tsunoda et al., 1980; Snogerup 1990). Most of the *B. oleracea* crops are serve as a good source of protein and carotenoids (Kopsell and Kopsell, 2006). In addition, studies have shown that *B. oleracea* contain also diverse phytochemicals (glucosinolates, GSLs) for plant defence against fungal and bacterial pathogens (Halkeir et al., 2006) and anticancer properties (Khwaja et al., 2009).

Kingdom: *Plantae* - Plants

Subkingdom: *Tracheobiota* - Vascular plants

Superdivision: *Spermatophyta* - Seed plants

Division: *Magnoliophyta* - Flowering plants

Class: *Magnoliopsida* - Dicotyledons

Family: *Brassicaceae* - Mustard family

Genus: *Brassica* L. - mustard

Species: *Brassica oleracea* L. - cabbage

Subspecies: *acephala* D.C. - kale

alboglabra (L. H. Bailey) Musil - Chinase kale, Chinase broccoli

botrytis L. - cauliflower

capitata L. - head cabbage

gemmifera DC. - Brussels sprouts

gongylodes L. - kohlrabi

italica Plenck - broccoli

medullosa Thell. - marrow-stem kale

oleracea L. - wild cabbage

ramosa DC. - perennial kale

viridis L. - collards

sabellica L. - curly kale, feathered cabbage

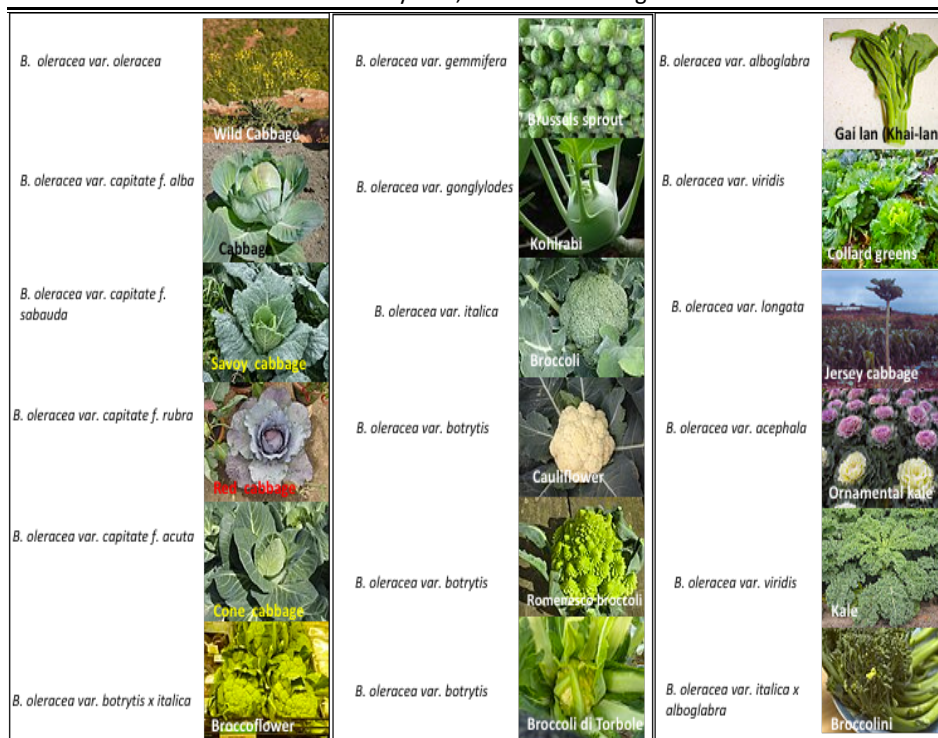


Figure 1.0: Taxonomy of the *B. oleracea* species (Source: NCBI Taxonomy Brower; www.ncbi.nlm.nih.gov/Taxonomy) and Pictures of some selected *B. oleracea* cultivars.

1.2.1 The Habitat of wild *B. oleracea* species

The naturally occurring environment of *B. oleracea* wild uncultivated species has been considered to be the areas of high salt and lime (Snogerup et al., 1990). They are often found in limestone sea cliffs, which is attributed to its intolerance of other plants (Snogerup et al., 1990). The adaptation of wild *B. oleracea* to the harsh environment has been related to its morphological advantage. The tall biennial plant of *B. oleracea* has shown to form a stout rosette of larger leaves in their first year. The description of their leaves has shown that they are fleshier and thicker as compared to the other *Brassicas*. These offer an adaptational advantage to store more water and nutrients under harsh growing environment. It also shows the ability to utilise stored nutrients to produce flower spike and flowers (Gray, 1980).

Moreover, morphological variation among the wild populations of *B. oleracea* species has been reported. Studies have shown that there is considerable morphological variation in both intra- and inter-population in wild species. The morphology of wild *B. oleracea* population differ significantly based on their vegetative and generative characters (McNaughton, 1995a) (**Figure 1.1**).



Figure 1.1: Different plants morphology in some selected diversified wild *B. oleracea* species

1.2.2 The significance of morphological diversity and *B. oleracea* crops domestication

Studies reveal the potential of *B. oleracea* species particularly in morphological evolution complementing what is obtainable in *Arabidopsis thaliana* (Lan et al., 2000; Bowman, 2007). Morphological divergence in *B. oleracea* has been relatively unusual due to its rapid reproduction isolation. That's is to say, a single species with a stunning range of morphologies among the genotypes can be readily intercrossed (Wang et al., 2011). Domestication of many plant crops has resulted in the enhancement of plant parts for use humans, such includes; the seeds, grains of cereal crops, the fruits of some trees, or roots in some vegetable crops. The *B. oleracea* crops are of exceptional in their range of striking morphology (Wang et al., 2011). They have been selected based on their vegetative meristems, for instance, cabbages for enlarged vegetative apex meristems, Brussels sprouts for leaf axils, broccoli for proliferation of floral meristems, cauliflower for aborted floral

meristems, kohlrabi, and kales for swollen bulbous stems and orate leaf patterns respectively (Wang et al., 2011). (**Figure 1.0**).

1.2.3 Cytological relationship and botanical description of *B. oleracea* and other members of *Brassicaceae* and *Arabidopsis* model plant

B. oleracea belongs to genus *Brassica* in the tribe *Brassicaceae*, a well-defined clade in the family of Brassicaceae that also includes *A. thaliana*. This was the first flowering plant to have its genome sequenced (AGI 2000). A comparative analysis study based on mitochondrial DNA has revealed that *Brassica* and *A. thaliana* lineages shared an ancestral ancestor ~14-20 million years ago (MYA) (Yang et al., 1999; Koch and Mummenhoff, 2006). Before their divergence, record shows that one round of ancient whole-genome triplication (WGD) referred to as gamma event (γ) in an early eudicot ancestor and two WGD i.e., alpha and beta events (α & β). This is thought to have occurred before the splitting of Arabidopsis-Brassica (Lan et al., 2000; Schmidt et al., 2001; Tang et al., 2008). Further additional polyploidisation(s) in Brassica lineage has been reported to occur after its divergence from Arabidopsis, this reflected by large segments of duplication in the genetic maps of the three diploids; *B. rapa*, *B. nigra* and *B. oleracea* (Parkin et al., 2003; Lukens et al., 2004). Arabidopsis and Brassica share physiological and biological similarities. Comparative genomics studies with the brassicas have been suggested as a means for the identification of genetic determinants in a more broader spectrum of variation than what may be obtainable using Arabidopsis alone (Lan et al., 2000; Bowman, 2007).

The relationships among the cultivated *Brassica* species have been described by the use of cytological studies (Morinaga 1934). According to these studies, the *B. oleracea*, *B. rapa* and *B. nigra* have been represented as three species of genus *Brassica* with C, A and B

genomes, which differ in their chromosome number and genome size. The number of chromosomes; i.e., $n = 16$, $n = 18$, and $n = 20$ was described for *B. nigra* (B-genome), *B. oleracea* (C-genome), and *B. rapa* (A-genome) respectively. These species underwent interspecific hybridisation and duplication event that led to the formation of allotetraploids (amphidiploids) of *B. napus* ($2n = 38$, AACCC), *B. juncea* ($2n = 36$, AABB), and *B. carinata* ($2n = 34$, BBCC). The genome size of diploid species has been characterised by low nuclear DNA contents. Studies have shown that the genomes range between 0.97 pg/2C (468 Mb/1C) for *B. nigra* to 1.37 pg/2C (662 Mb/1C) for *B. oleracea* (Arumuganathan and Earle, 1991). Also, within the *B. oleracea* species, the record has shown that the size of the genomes varies from 599 Mb/1C in broccoli to 662 Mb/1C in cauliflower respectively.

The phylogenetic and genome relationships between diploids and amphidiploids have been described and illustrated by using the U's triangle (1935) and further confirmed by using cytogenetic studies (Prakash and Hinata, 1980) (**Figure 1.2**).

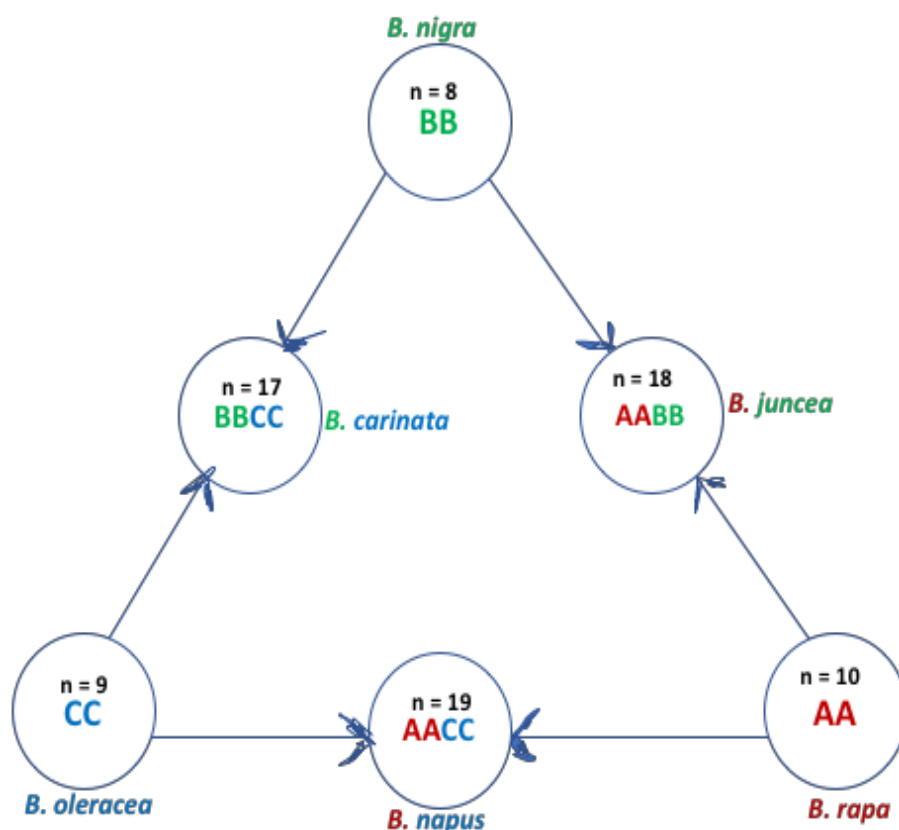


Figure 1.2: The U's Triangle showing the phylogenetic relationships between *Brassica* species (n = haploid number of chromosomes, and **AA**, **BB**, **CC** and **AABB**, **AACC** and **BBCC** indicate genome). (Morinaga 1934; U 1935).

1.2.4 History and Origin of wild *Brassica* species

The wild taxa of the species (**Table 1a & 1b**) have a global distribution with a specific presence in many regions of the world, e.g., in the Mediterranean region and areas along the Atlantic coasts. Findings have suggested that the *Brassic*as evolved from the genus *Sinapidendron* of the Miocene age through *Diplotaxis Erucastrum* complex (Gomez-Campo and Tortosa 1974). The cultivated *Brassica* species are also described by using two way systems, first, with a possibility of *B. nigra* being derived from the *Sinapis* stock and second, *B. rapa* and *B. oleracea* developed from *Diplotaxis eruroides* (Song et al., 1990; Warwick and Black, 1991; Pradhan et al., 1992). Reports have shown that the first *Brassica* species to be domesticated was *B. rapa*; this was attributed to the closeness of its natural area to the centre of domestication, which has extended from Mediterranean region to

Central Asia in ancient times. Two main domestication centres for *B. rapa* have been reported; one in Europe, which represent the *oleiferous* turnip centre, and China, which is represented by the leafy form *pak choi* (Song et al., 1988b) centre of origin of *B. nigra*. This overlaps with *B. rapa* thus both wild-type of *B. rapa* and *B. nigra* occur together in the Middle East, and hence, it is believed that *B. juncea* might have originated there. Other secondary centres considered for *B. juncea* were China and India (Song et al., 1988). The genus *B. oleracea* developed much later and is largely far away from the centre of domestication (Gomez-Campo, 1999).

The members of *B. oleracea* species are botanically diverse, they share considerable variation both in character and shape. These have been observed in many regions, for example, the East Mediterranean and Sicily (Snogerup, 1980). *Brassica hilarionis* is dominant around Kyrenia mountains, Cyprus, while *B. cretica* L. are widely distributed in some part of Greece and Turkey. The *B. incana* populations are distributed in many Island, for instance, north-eastern part of Sicily, while *B. rupestris* R. and *B. villosa* are found mostly from the western part of Sicily. Moreover, wild *B. macrocarpa* has been reported in both islands of Sicily and *B. insularis* (Moris) are present in Sardinia, Corsica, and Tunisia respectively. *B. montana* (Pourret) grow naturally around northern Italy, southern France, and Spain (Padilla et al., 2005). In addition, the wildtype of *B. bourgeai* species grows in the Canary Islands. Wild populations of *B. oleracea* crops are found in areas around the Atlantic Coasts of northern Spain, France, Great Britain, and Helgoland (Gustafsson and Lannér-Herrera, 1997).

Table 1a: Genomic designations of varietal or subspecific taxa of agriculturally important brassicas. Considerable taxonomic confusion exists in the literature for *Brassica* (Toxopeus et al., 1984). These designations are a convenient classification of the major Brassica morphotypes. n, the haploid complement of chromosomes; a, 10; b, 8; c and r, 9.

Species (n)	Subspecies or variety	Genome descriptor (2n)	Common Name
<i>Brassica</i>			
<i>nigra</i> (8)		bb	Black mustard
<i>oleracea</i> (9)		cc	Cole crops
	<i>acephala</i>	cc.a	Kales
	<i>alboglabra</i>	cc.al	Chinis kale, Kailan
	<i>botrytis</i>	cc.b	Cauliflower, Heading broccoli
	<i>capitata</i>	cc.c	Cabbage
	<i>costata</i>	cc.co	Portuguese cabbage
	<i>gemmifera</i>	cc.g	Brussels sprouts
	<i>gongylodes</i>	cc.go	Kohlrabi
	<i>italica</i>	cc.i	Broccoli, Calabrese
	<i>medullosa</i>	cc.m	Marrow stem kale
	<i>palmifolia</i>	cc.p	Tree cabbage
	<i>ramosa</i>	cc.ra	Thousand-head kale
	<i>sabauda</i>	cc.s	Savoy cabbage
	<i>sabellica</i>	cc.sa	Collards
	<i>selensia</i>	cc.sc	Borecole
<i>campestris</i> (10)		aa	
<i>(syn. rapa)</i>	<i>chinensis</i>	aa.c	Pak choi
	<i>narinosa</i>	aa.na	
	<i>nipposinica</i>	aa.na	
	<i>oleifera</i>	aa.o	Turnip rape, Toria
	<i>parachinensis</i>	aa.pa	Choy sum
	<i>pekinensis</i>	aa.p	Chinese cabbage, Petsai
	<i>perviridis</i>	aa.pe	Tendergreen, Komatsuna, Mustard spinach
	<i>rapifera</i>	aa.r	Turnip
	<i>trilocularis</i>	aa.t	Sarson
	<i>utilis</i>	aa.u	Broccoli raab
<i>carinata</i> (17)		bbcc	Ethiopian mustard
<i>junceae</i> (18)		aabb	
	<i>capitata</i>	aabb.c	Head mustard
	<i>crispifolia</i>	aabb.cr	Cut leaf mustard
	<i>facilifera</i>	aabb.f	Broccoli mustard
	<i>lapitata</i>	aabb.l	Large petiole
	<i>multiceps</i>	aabb.m	Multishoot mustard
	<i>oleifera</i>	aabb.o	Indian mustard, Raya
	<i>rapifera</i>	aabb.r	Root mustard
	<i>rugosa</i>	aabb.ru	Leaf mustard
	<i>spicea</i>	aabb.sp	Mustard
	<i>tsa-tsai</i>	aabb.t	Big stem mustard
<i>napus</i> (19)		aacc	Fodder rape
	<i>oleifera</i>	aacc.o	Oil rape
	<i>rapifera</i>	aacc.r	Swede, Rutabaga

Source: Table was adapted from Williams and Curtis, (1986) *Science* **232**: 1385 – 1389.

Table 1b: Chromosome Numbers for the taxa of the tribe *Brassicaceae*

TAXON [Name Reported as]	n	2n	Reference
<i>Brassica bourgeau</i> (Webb ex H. Christ) Kuntze	9	18 (20)	Borgen et al. (1979)
<i>Brassica bourgeau</i>	-	18	Snogerup et al. (1990)
<i>Brassica cretica</i> Lam.	9	-	Griesinger (1937)
<i>Brassica cretica</i>	-	20	Miège and Greuter (1973)
<i>Brassica cretica</i>	9	-	Montmollin (1986)
<i>Brassica cretica</i> subsp. <i>cretica</i>	-	18	Snogerup et al. (1990)
<i>Brassica hilarionis</i> Post	-	18	Snogerup et al. (1990)
<i>Brassica incana</i> Ten.	9	-	Griesinger (1937)
<i>Brassica incana</i> [as <i>B. sylvestris</i> subsp. <i>sylvestris</i> (Lam.) Mill.]	9	-	Griesinger (1937)
<i>Brassica incana</i>	9	-	Takahata and Hinata (1978)
<i>Brassica incana</i>	-	18	Ferrarella et al. (1979a)
<i>Brassica incana</i>	9	-	Gómez-Campo and Hinata (1980)
<i>Brassica incana</i>	-	18	Snogerup et al. (1990)
<i>Brassica insularis</i> Moris	-	18	Manton (1932)
<i>Brassica insularis</i> [as <i>B. oleracea</i> subsp. <i>insularis</i> (Moris) Rouy & Foucaud]	-	18	Contandriopoulos (1957c)
<i>Brassica insularis</i> [as <i>B. oleracea</i> subsp. <i>insularis</i>]	-	18	Contandriopoulos (1962)
<i>Brassica insularis</i>	9	18	Corsi (1963)
<i>Brassica insularis</i>	9	-	Salmeen (1979)
<i>Brassica insularis</i>	9	-	Snogerup and Persson (1983)
<i>Brassica insularis</i>	-	18	Baltisberger (1988)
<i>Brassica insularis</i>	-	18	Lentini et al. (1988)
<i>Brassica insularis</i> [as var. <i>insularis</i> Moris]	-	27	Verlaque et al. (1993)
<i>Brassica macrocarpa</i> Guss.	-	18	Takahata and Hinata (1978)
<i>Brassica macrocarpa</i>	-	18	Ferrarella et al. (1979b)
<i>Brassica macrocarpa</i>	9	-	Gómez-Campo and Hinata (1980)
<i>Brassica macrocarpa</i>	-	18	Snogerup et al. (1990)
<i>Brassica montana</i> Pourr.	-	18	Netroufal (1927)
<i>Brassica montana</i>	9	-	Harberd (1972)
<i>Brassica montana</i>	-	18	Snogerup et al. (1990)
<i>Brassica oleracea</i> L.	-	18	Nagai and Sasaoka (1930a)
<i>Brassica oleracea</i>	-	18	Nagai and Sasaoka (1930b)
<i>Brassica oleracea</i>	9	18	Catcheside (1937)
<i>Brassica oleracea</i>	-	18	Richharia (1937a)
<i>Brassica oleracea</i> subsp. <i>oleracea</i>	-	18	Leveque and Gorenflot (1969)
<i>Brassica oleracea</i>	-	18	Mitsukuri (1956)
<i>Brassica oleracea</i>	9	-	Takahata and Hinata (1978)
<i>Brassica oleracea</i>	9	-	Lan (1986)
var. <i>alboglabra</i> (L.H. Bailey) [as <i>B. alboglabra</i> L.H. Bailey]	-	18	Nagai and Sasaoka (1930a)
var. <i>botrytis</i> L.	-	18	Karpetchenko (1924)
var. <i>capitata</i> L.	-	18	Karpetchenko (1924)
var. <i>italica</i> Plenck	-	18	Murin (1978)
var. <i>ramosa</i> DC.	-	18, 36	Zeven et al. (1989)
<i>Brassica rupestris</i> Raf.	-	18	Manton (1932)
<i>Brassica rupestris</i>	9	-	Harberd (1972)
<i>Brassica rupestris</i>	-	18	Colombo et al. (1978)
<i>Brassica rupestris</i>	-	18	Takahata and Hinata (1978)
<i>Brassica rupestris</i>	9	-	Salmeen (1979)
<i>Brassica rupestris</i>	-	18	Snogerup et al. (1990)
<i>Brassica villosa</i> Biv.	-	18	Raimondo et al. (1980)
<i>Brassica villosa</i>	-	18	Snogerup et al. (1990)

* **Note:** The Table was adapted from a draft of checklist containing all known reports of haploid (*n*) and diploid chromosome numbers (*2n*) for taxa of the tribe *Brassicaceae* as contained in Guide to Wild Germplasm of Brassica and Allied Crops (Tribe *Brassicaceae*, *Brassicaceae*) Part II.

1.3 Salt Stress

1.3.1 Salt Stress as a factor affecting the environment

Under normal field growing conditions, plants are exposed to various environmental factors, which mostly constitute their immediate environment and how they relate symbiotically with the ecosystem. A deviation from the optimal environmental level causes deleterious effects to plant growth and lead to a situation referred to as stress conditions. To define the term stress condition two technical terms have been used: (A) By using **physical terms**, stress is defined as applied mechanical force per unit area of an object. Thus, by using this definition, it would be difficult to measure exact applied force as a result of stress because plants are considered to be perpetual. (B) To define stress in **biological terms** could also be conditional because a condition seems to act a stress relative one plant may be an ideal for another plant (Hernandez et al., 1995). Therefore, the biological definition of stress has been any harsh state/conditions or 'applied force' that impedes the normal functioning of a biological system such as plants (Hernandez et al., 1995).

However, different sources of plants stress have been identified and categorically fall into two factions; abiotic factors; for instance high temperatures, cold, drought, salt stress (salinity), and biotic factors; as a result of infection from viruses, insects, nematodes, bacteria, and fungi have been reported to affect the extent of production and cultivation of agricultural crops, and amongst these stresses, salt stress has been argued to be the most important environmental factor limiting crop productivity and quality (Zhu, 2001; Tester and Davenport, 2003; Yamaguchi and Blumwald, 2005; Shahbaz and Ashraf, 2013).

A saline soil is generally defined as any soil with electrical conductivity (EC) of the saturation extract (ECe) in the root zone exceeding 4 dSm^{-1} , which is equivalent to 40 mM NaCl at 25°C and has an exchangeable sodium of 15% (Munns, 2005; Jamil et al., 2011). At this ECe, many crops yield have shown to be reduced or affected (Jamil et al., 2011).

That is to say, increase soils salinity is the main causes of salt in the soil, therefore salt stress is an environmental factor caused by the increase of salinity and the effects usually refer to as salt stress or salinity stress.

1.3.2 Soils Salts and its Sources

The global scarcity of many valuable resources including water, environmental pollution and increased salinization of soil water has been marked as prominent challenge to the 21st century, because of growing number human population and reduction in the availability of arable land for crop cultivation to meet the need for agricultural sustainability (Shahbaz and Ashraf, 2013). However, soil salinity was first reported in Tigris-Euphrates alluvial plains of Iraq (Russel et al., 1965). The salt-affected soils have shown to affect large climatic regions worldwide, found in different latitudes and in places such as plateaux and the rocky mountains (Singh and Chatrath, 2001). Dissolved salts have shown to be present in all soils, in irrigated waters from canals and underground water (Hanson and May 2011). In fact, the integral part of the earth crust has been formed by these salts and they form chemically complex compounds with essential nutrients for plants growth and developments (example, nitrates, Calcium, Magnesium and Potassium salts) (Singh and Chatrath, 2001).

The major sources of soil salinity have been from mineral weathering, inappropriate use of inorganic fertilizers, gypsum, composts and, organic manures and irrigation waters (Katuby-Amacher et al., 2000). Unguided irrigation of crop plants using contaminated water, in particular, has shown to encourage soil salinity. Other implicated sources are unhealthy agricultural practices and the overall effects are the deposition of large amounts of salts to the soil (Munns, 2002). The mineral content of irrigated water includes salts of calcium (Ca^{2+}), magnesium (Mg^{2+}), and sodium (Na^{+}) (Munns, 2002). Therefore, evaporation of

surface water due to high temperature and drought causes Ca^{2+} and Mg^{2+} to precipitate into anhydrous carbonated form leaving only sodium salts to dominate the soil thereby increasing the Na^+ concentrations to supersede other macromolecules and micronutrients nutrients. High Na^+ level in the soil deprive plants of other essential nutrients through complex chemistry via interference of nutrient-ion activities and produced extreme $\text{Na}^+/\text{Ca}^{2+}$ and Na^+/K^+ ratios (Grattana and Grieveb, 1999; Hanson and May 2011).

1.3.3 Impact of Salinity Stress on Plants Growth

Salinity stress effects are the results of complex interactions involving processes associated with morphological, physiological, and biochemical involvement that affects seed germination, growth, and water and nutrient uptake (Akbarimoghaddam et al., 2011; Jung and McCouch, 2013). Soil salinity has shown to impose ion toxicity (**Figure 1.3**), osmotic stress, nutrients deficiency and oxidative stress on plants thereby limiting their overall water uptake (Munns, 2002). Excessive accumulation of elements such as sodium, chloride, and boron have shown to cause specific effects on plants. Sodium accumulation causes cell walls distortion, which can lead to osmotic stress (**Figure 1.3**), and cell death (Munns and Tester, 2008). Salinity also affects photosynthesis mainly through a reduction in leaf area, chlorophyll content and stomatal conductance, and to some extent through a decrease in photosystem II efficiency (Netonda et al., 2004; Munns and Tester, 2008). Also, reproductive development has shown to be affected by salinity stress through inhibitory effects on microsporogenesis and stamen filament elongation, promoting programmed cell death, ovule abortion and senescence of fertilized embryos (Ashraf, 2004). All these factors cause adverse effects on plant growth, and development at physiology, and biochemical levels and molecularly (Munns and James, 2003; Tester and Davenport, 2003).

Assessing tolerance of plants to salinity stress, growth, and survival. Salt tolerance in a plant is defined as plant's ability to reproduce under salt conditions and improve in percent biomass in comparison to a healthy (unsalted) growing environment in an extended period (Prasad et al., 2016). Significant variations have been reported in plants response as a decline in growth is observed often exasperated by the length of exposure over which plants are expected to grow in saline-affected soils (Prasad et al., 2016).

Many studies have shown that the percent decrease in biomass growth has a direct relation with the degree of tolerance to salt. For example, a salt-tolerant plant such as sugar beet has shown to undergo a 20% decrease in total dry weight when exposed to 200 mM NaCl (Gorham and Jones, 2002). However, it was observed that when a plant with moderate salt tolerance is grown in the same salt concentrations such as cotton, a 60% reduction in total dry weight was reported (Gorham and Jones, 2002). A case study using a halophyte plant, *Suaeda maritima* shows that at 200 mM NaCl conditions, the plant reached its full growth (Flowers and Yeo, 1986). Evaluation of different plant species for their salt-tolerance has been based on their ability to survive. For instance, a perennial plant has been classified tolerant based on their survival rate. A decline in growth rate under salt conditions has been observed in both salt-tolerant and nontolerant plant species after a short exposure to salt stress, because sudden and moderate exposure to soil salinity causes leaf cells to lose water and also inhibits lateral shoot development, and symptoms begin to appear over weeks of growth but this more to salt-sensitive plants than salt tolerant (Munns and Tester, 2008) .

1.3.4 Physiological Models Describing Plant Response to Salt Stress Conditions

The reaction toward salt stress by plants has been represented by using two mechanistic phases: the primary phase, referred to as osmotic phase; and the second phase, usually referred to as the ionic phase (Munns and Tester, 2008) respectively (**Figure 1.3**).

The experience for plants suddenly exposed to salt stress has been an osmotic shock (stress), which causes a reduction in leaf expansion (Munns, 1993). The osmotic effects have been regarded as an immediate response by plants on the sudden onset of salt experience (**Figure 1.3**). This has been shown to continue for a period and could last throughout the exposure, thus triggering a reduction in cell expansion, division, and as well as the stomatal closure (Munns, 2002; Flowers, 2004). The prolonged exposure to salinity could lead to ionic stress, which causes premature senescence of adult leaf, and a reduction in photosynthesis. The dissociations of NaCl salt would produce excess sodium ions and chloride ions. The excess Na^+ ions have the potential to affect the enzymes activities in the plants. The chloride ions play important role in anion homeostasis but an excess of it could be toxic to plant growth. Excesses of Na^+ and Cl^- have been shown cause cell swelling, lead to energy reductions and other physiological changes such as; premature senescence, disruption of protein synthesis, which could further interfere with enzymes activities (Hasegawa *et al.*, 2000a; Munns, 2002). Different mechanisms of resistance have been reported to be employed by plants. For example, plants may either exclude salt from their cells and second, tolerate its presence within the cells to prevent its toxic effects. The mechanisms involved in response to salt stress by plants include; a) biochemical response (b) molecular response, and (c) tissue tolerance (Munns and Tester, 2008).

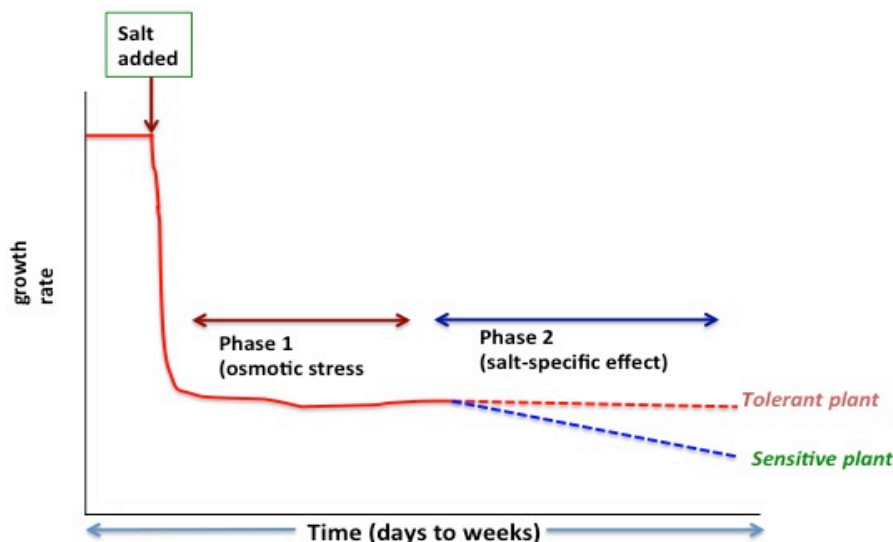


Figure 1.3: Physiological Models describing two-phase growth response by the plant under salinity conditions. A two-phase model proposed (Munns et al., 1995), describing the osmotic and ionic effects of salt stress. Plants that are sensitive or tolerant to salinity may differ in the rate at which salt reaches many sites of the plant's body; in roots, shoots, or leaves. Timescale to response to salt could be hours, days or weeks or months, depending on the species and salinity level. In **Phase 1**: the plants may experience an early reduction in growth, causes attributed to osmotic effects of an increased salt solution outside the roots. **Phase 2**: they may experience increasing dead of old leaves in case of the sensitive plants and reduction in photosynthetic capacity of the plant, hence, triggered biochemical, physiological and molecular adjustments.

1.3.5 Plant Response to Salinity Stress

The effects of salt stress on plant differ mainly due to their genetic differences. The mechanisms involved also differ due to their nature, the complexity of the interactions between environment and genetic make-up, all of which could affect the plant's phenotype responses. The mechanisms of genetic control of salt tolerance in plants are complex. Several genes have been reported to participate in the salt stress tolerance in different species and these depend strongly on interaction/environmental conditions (Allen et al., 1994). The determination of salt stress by genetic variation has been determined through indirect approaches measuring responses of different genotypes, mainly through assessing their growth and yield under different salt conditions (Allen et al., 1994). Percent biomass has

been reported in many studies comparing the control plant and treated under a controlled environment over a period, while tolerance measured as survival has been used mainly for perennial species (Munns, 2002).

Salt tolerance has shown to vary considerably. It has been shown that their response to sudden exposure to salinity affects the way halophytes responds (Albert, 1975). Differential adaptive mechanisms have been hypothesised to be involved including, a gradual acclimation to salinity in contrast to adjustment, and to a sudden exposure (Albert, 1975).

Different plants have evolved several mechanisms to improve or acclimatise to salinity. These include primary mechanisms including osmotic tolerance, the regulation of signal distribution, shoot growth and reduction in young leaves production. These are triggered as initial effects before Na^+ becomes accumulated in the shoot and the toxic stage is reached. Secondary mechanisms include ion exclusion, which has shown to involve regulation of Na^+ and Cl^- ions influx to prevent over-accumulation and to prevent toxic concentrations within the leaves. And thirdly, tissue tolerance, which involves compartmentalization of accumulated salt in leaves into cellular and intracellular spaces (mainly in the vacuole) (Munns and Tester, 2008).

1.3.6 Plant Salt Stress Signalling Pathways

The initial uptake and the net influx of Na^+ may be dependent upon the plant's previous exposure to salt, which could result in significant flow initially, but is reduced when the level of exposure increases. Chen et al., (2007) reported that sodium uptake varied between 10 – 20 min when moderate or high external Na^+ are applied. Critical mechanisms regulate the movement of Na^+ in and out of the cell, and these rely on input signals from secondary

messengers such as calcium (Ca^{2+}), cyclic guanosine monophosphate (cGMP), and reactive oxygen species (ROS). Due to elevated sodium concentration, increased ROS production have been reported in the cytoplasm (Knight et al., 1997; Kiegle et al., 2000; Donaldson et al., 2004).

1.3.6.1 Ca^{2+} Signalling

Ca^{2+} , a divalent cation, and an essential plant nutrient that plays a vital role mainly in structure formation in the cell wall and membranes. It act as the principal cation in a counter-cation pump for both organic and inorganic anions in the cell vacuole. It also serves as a secondary messenger, an increase in the cytosolic concentration due to developmental and environmental challenges and its coordination role in responses have been reported (Marschner, 1995). It is one of the elements that are rarely deficient in plants and is readily absorbed from soils. As the abundant cation Ca^{2+} , concentration in the cytoplasm has usually been kept low by sequestration in the vacuoles, also by forming complexes with calcium-binding proteins, e.g., calmodulin, or through precipitation as calcium oxalate crystals (Bush, 1995; White and Broadley, 2003). It's roles as secondary messenger coordinate activities within the cell. It also has a role in stimulus-response coupling in signal transduction pathways, as it has been reported to facilitate an intracellular release of Ca^{2+} from its pool stimulates many protein kinases, phosphatases, and phospholipases, which shown to have a downstream targets molecules in cellular functions (Bush, 1995).

Plant cells are shown to absorb calcium through Ca^{2+} -permeable channels present in their membranes (White, 2000). High concentrations of calcium have been reported to be toxic, and thus a relatively low level is been ensured by the activities of Ca^{2+} -ATPases and $\text{H}^+/\text{Ca}^{2+}$ -antiporters. These antiporters ensure the removal of excess cytoplasmic Ca^{2+} to

apoplast and the lumen of intracellular organelles, e.g., vacuole and endoplasmic reticulum (ER) (**Figure 1.5**) (Sze et al., 2000; Hirschi, 2001). The cytosolic Ca^{2+} accumulations have been implicated to serve as a signal to a diverse range of cues due to developmental and environmental challenges. Reports have shown that rapid influx of calcium ions via cation channels in the plasma membrane, tonoplast, and ER has a direct relationship with generated perturbations, which could lead to significant cellular responses from different environmental stresses, e.g., abiotic (White, 2000; Sanders et al., 2002). A Series of enzymatic reactions involving protein-conformational changes and catalytic activity are some of the vital events that could affect cellular perception and transduction of the calcium ion signal. Protein binding could involve calmodulin (CaM), calcineurin B-like proteins (CBLs) and calcium-dependent protein kinases (CDPKs). It has been hypothesised that responses due to cellular accumulation of calcium have been examples of specific stimuli by biotic and abiotic encoded by cytoplasmic calcium perturbations that are transduced by calcium sensors individually to respond positively through undisclosed networks (White and Broadley, 2003).

1.3.6.2 Cyclic Guanosine Monophosphate (cGMP) Signalling

A study by Donaldson et al., (2004), have shown an upward increase in cGMP increases as a result of the onset of salt and osmotic stresses (Figure 1.4). Maathuis and Sanders (2001); Essah et al., (2003) have corroborated same observation using *Arabidopsis* seed. Also, a study by Rubio et al., (2003) have described how the influx of Na^+ is affected by the level of cGMP. The inhibitory role of cGMP on membrane-bound non-selective cation channels was further demonstrated in many studies involving *Arabidopsis* root protoplast (*AtKTI*) (reviewed: Maathuis and Sanders, 2001).

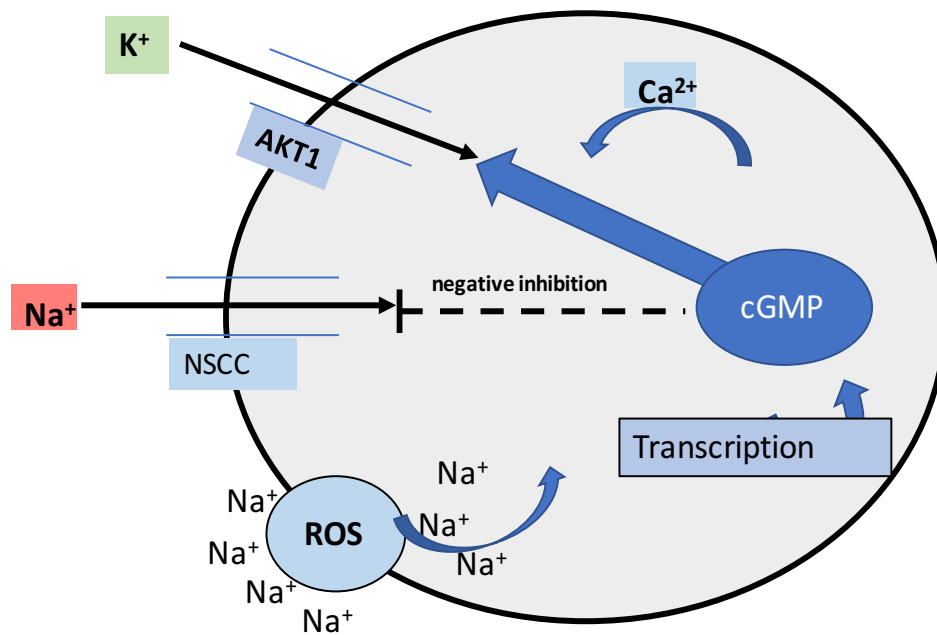


Figure 1.4: A mechanistic transient increase in secondary messengers Ca^{2+} , $cGMP$ and ROS due to osmotic and ionic stresses. $cGMP$ regulates Na^+ influx by negatively inhibiting the non-selective cation channel (NSCC) and facilitating potassium uptake via inward rectifying K^+ channel (AtKT1) in Arabidopsis and downregulation of Na^+ absorption due to the downstream effect of transcription processes (Rubio et al., 2003).

The study further indicates that an external application of $cGMP$ had an inhibitory effect on net uptake of Na^+ and promoted K^+ uptake and affect transcript levels of genes, specifically those related to transmembrane proteins (Maathuis, 2006). The presence of calcium ions has shown to have an impact on the action of $cGMP$. It has shown to serve as signalling intermediary downstream to $cGMP$ signal (Donaldson et al., 2004).

1.3.6.3 Reactive Oxygen Species (ROS)

The role of reactive oxygen species (ROS) in the regulation of Na^+ influx has been attributed to the osmotic effect of salt stress, which has been shown to promotes the release of its products, hydrogen peroxide (H_2O_2) and superoxide anions (Miller et al., 2010). In addition, up-regulation of antioxidant enzymes, i.e., superoxide dismutases, catalases, and

peroxidases have shown to frequently soften responses as the generation of antioxidant proteins have been reported to serve as signals (Hong et al., 2011). The generation of ROS under salt stress has been shown to occur within minutes and to involve mainly H₂O₂, which depends on the activity of NADPH oxidases (Hong et al., 2011). Other studies have also shown that specific ROS-sensitive transcription factors could be activated such as ethylene response factor 1 (ERF1), which have multiple effects on promoters downstream including mitogen-activated protein kinase cascades (MAPK) (Schmidt et al., 2013).

1.3.6.4 Salt Overly Sensitive Pathway (SOS)

Meanwhile, the effluxes of Na⁺ ions from the cytoplasm to either apoplast or to be compartmentalised in the vacuole have been reported in association with salt overly sensitive (SOS) pathway (**Figure 1.5**). Studies have identified SOS1 as antiporter bound to the plasma membrane Na⁺/H⁺ antiporter that ensures the extrusion of Na⁺ from the cytoplasm and has been described as the best mechanism to salt stress tolerance (Chinusamy et al., 2004). The activity of SOS1 is associated with the action of kinase enzymes calcium-induced protein kinase family CIPK24 (SOS2), which have been reported to phosphorylate the SOS1 (Chinusamy et al., 2004). SOS2 reported being active as a result of an association with another calcium-dependent protein calcineurin-B like-4 (CBL4) also known as calcium sensor CBL4 (SOS3; **Figure 1.5**) (Chinusamy et al., 2004).

The activation of SOS1 involves a series of reactions that have to do with the bindings of Ca²⁺ to the regulatory site of SOS3, which causes dimerization of the catalytic site of SOS3, which further ensure associations and interactions with the amino acids domain of SOS2, thereby activating it. The Complex intermediary that has shown to form SOS3-SOS2-complex (**Figure 1.5**), mediates the phosphorylation reaction at the C-terminus of SOS1

leading to the removal of SOS1 inhibitory domain, therefore further activating the antiporter (Quintero et al., 2011). Activation of SOS1 antiporter has shown to increase the efflux of Na^+ from the cytoplasm to vacuole thereby reducing its accumulation (Quintero et al., 2011). The activated form of SOS2 has been implicated to affects the activity of other membrane transporters. And thus, involve in Na^+ uptake like high-affinity potassium transporters (AtKT1) in *Arabidopsis*. Therefore, via an interaction between SOS2, and vacuolar Na^+/H^+ antiporter an isoform discovered in Arabidopsis plant (AtNHX1) have been reported to reduce the cytoplasmic accumulation of Na^+ , where AtNHX1 has been shown to be the significant Na^+ evacuator into the vacuole (Apse et al., 1999; Rus et al., 2006; Laurie et al., 2002).

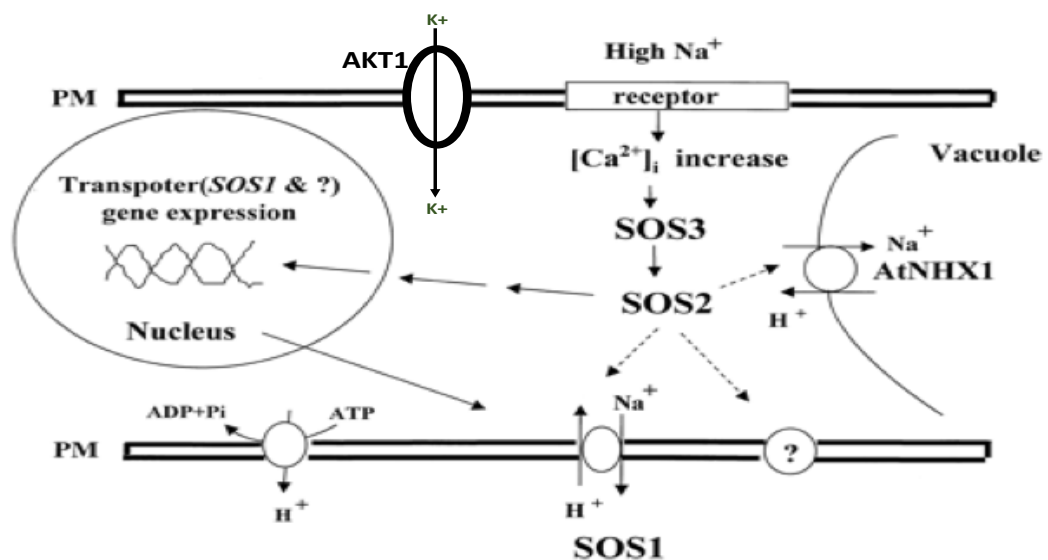


Figure 1.5: Mechanism of Na^+ extrusion at the plasma membrane is an active process mediated by Na^+ -ATPases. At acidic extracellular pH, Na^+ extrusion at the plasma membrane is mediated mainly Na^+/H^+ antiporter (*SOS1*) activated by a complex (SOS2-SOS3) upon binding by calcium. Also activated by this complex is high-affinity potassium transporters (*AKT1*) discovered in *Arabidopsis* which allow potassium entrance into the cytoplasm. (SOS2-SOS3) the complex may facilitate the expression of transcriptions factors that have a downstream effect. *AtNHX1*, a vacuolar Na^+/H^+ antiporter discovered in *Arabidopsis* plant. This gene expression and transporter activity regulation brings about homeostasis of ions such as Na^+ and K^+ and consequently plant tolerance to Na^+ stress (Zhu, 2001).

1.3.7 Phases of Plant Response to Salt Stress

1.3.7.1 Osmotic Phase

The osmotic phase has been described to involve mechanisms that are mainly regarded as to be rapid, long-distance distribution of signalling, including the processes of ROS generation, Ca^{2+} waves and electrical signalling (Mittler et al., 2011). Although importation of osmotic tolerance is little known, reports have shown that it causes a reduction in the growth of a salt-stressed plant. Regardless of the plant's ability to exclude salt, the osmotic phase creates a decrease in growth rates due to a reduction in stomatal conductance (Fricke et al., 2004; James et al., 2008). Osmotic tolerance has been reported to involve plant's ability to stand the drought phase that was shown to be imposed as a result of salt stress, and its ability to retained leaf area expansion and increase stomatal conductance (Rajendran et al., 2009).

Osmotic tolerance has been reported to be affected by differences in the plant's ability to excite and maintain the long signal, and in the initial perception of the salt and differences in response to the signals. It has shown in a case study on different durum wheat varieties and landraces that tolerance to osmotic stress is positively correlated between the parameters of stomatal conductance and relative growth rate (James, 2008). The biochemical explanation has been attributed to the reduction in the comparable growth and ability of the plants to overcome the toxic level of salt. Toxic salt level has shown to affect the survival of older leaves, and eventually plant death because photosynthesis export can no longer be able to support by the younger leaves thereby affecting both growth and production (Munns and Tester, 2008). Additionally, increase in tolerance to osmotic stress has been associated with and characterised by the ability plant to improve both production and growth of fresh

and older leaves (leaf area), and higher stomatal conductance (Hu et al., 2007; Munns and Tester, 2008).

1.3.7.2 Ionic Phase

This phase has been shown to be involved in the movement of Na^+ and Cl^- ions which have been attributed to the mechanisms mainly for Na^+ exclusion from the cytoplasm by compartmentalisation intracellularly into the vacuole. This process has been described as a remedy to toxicity, thereby causing the plant to respond by using either one of the two mechanisms. Firstly, preventing the accumulation of ions to reach toxic concentration mainly in the leaf blades (Na^+ and Cl^- exclusion); the second one has to do with increasing ability to tolerate the salts by compartmentalisation process. These mechanisms have been reported widely to affect a range of plasma membrane-bound transporters and channel proteins that form part of the membrane and tonoplast (Tester and Davenport, 2003; Plett and Moller, 2010).

There has been significant genetic variation reported between halophytes and glycophytes toward the mechanism of Na^+ exclusion (Yoo *et al.*, 2009). Accumulation of Na^+ and Cl^- for osmotic adjustment in the saline environment has been reported in some halophytic plants especially C3 plants species (Yoo et al., 2009) The reports have indicated that Na^+ ions cause more toxicity in the initial stage before Cl^- does in plants under salt conditions, as such, most studies have been focussed more on Na^+ exclusion and its transport (Munns and Tester, 2008). The majority of plant species have been reported to have evolved essential mechanisms of salt tolerance through evolution. The ability of the plant to reduce its ionic stress has to do with an efficient mechanism to minimise the level of Na^+ in the cytosol of the cell, particularly in the transpiring younger leaves (Munns and Tester, 2008).

It has been hypothesised that halophytes can reduce both transpiration in the leaves and shoot Na^+ accumulation via an efficient intracellular sensing mechanism (Robinson et al., 1997; Very et al., 2014). Studies have revealed, some glycophytes such as; rice, durum wheat, bread wheat, and barley possess mechanisms for Na^+ exclusion associated with salt tolerance but the effectiveness of the mechanism differs from that of halophytic plants (Richard et al., 2010).

Transporting Na^+ through different parts of the plant from roots cortex to the shoot is tightly regulated. The exclusion of Na^+ from the leaves could be attributed to net Na^+ uptake by cells in the root cortex and has shown to control to the net loading into xylem by parenchyma cells (Davenport et al., 2005). The process of Na^+ exclusion by roots ensured Na^+ removal and delay its toxic concentrations within the leaf. Failure in any of these processes of Na^+ expulsion have been implicated to the manifestation of deleterious effects of toxic Na^+ on the plant species, and to lead to the early death of leaves especially the older leaves (Munns and Tester, 2008).

A number of membrane bound-transporters have been reported and suggested to be involved in efficient Na^+ exclusion and compartmentalisation both into the apoplast and vacuole (Tester and Davenport, 2003). The nature of this transport system is it utilises energy generated from the hydrolysis of ATP and inorganic phosphate (PPi) (Deinlein et al., 2014; Roy et al., 2014). Reported studies have shown that the effectiveness and efficiency of cytosolic Na^+ evacuator mechanism are dependent upon the activity of an antiporter, Na^+/H^+ exchanger (Deinlein et al., 2014; Roy et al., 2014). The activity of membrane-bound vacuolar proteins have been shown to move the potentially toxic ions from the cytosol into internally acidic, tonoplast-bound vacuoles (Tester and Davenport, 2003; Deinlein et al., 2014; Roy et al., 2014). The significance of this active transport of excess ions into vacuoles

has been described as “panacea” which serves as an osmoticum within the vacuole, which could help to maintain water flow into the cell, and thereby allowing the plant to grow in soils containing high salinity (Tester and Davenport, 2003; Deinlein et al., 2014; Roy et al., 2014). The electrochemical energy informs of proton-motive force generated through the activities of vacuolar H^+ -translocating enzymes, H^+ - adenosine triphosphate (ATPase), and H^+ - inorganic pyrophosphatase (PPiase) have shown to be used by antiporters to facilitate the downhill movement of H^+ (down its electrochemical gradient) with an upward movement of Na^+ (against its electrochemical gradient) Deinlein et al., 2014; Roy et al., 2014). Many studies have identified different isoforms of Na^+/H^+ antiporters, for example, *AtNHX1*, in *Arabidopsis* have been indicated to be involved in the control of vacuolar osmotic potential (James et al., 2006). Other examples such as *Nax1* and *Nax2* genes found in durum wheat genotype. Glycophytic plants have been reported to have higher affinity to K^+ over Na^+ and overexpression of *Nax1* and *Nax2* improve Na^+ exclusion from the roots-shoot and retention of Na^+ in the leaf (James et al., 2006). In another report, *the Nax2* gene has also been shown to enhanced Na^+ elimination and retention of K^+ through its power of discrimination, more affinity to K^+ over Na^+ (James et al., 2006). The mechanisms of Na^+ exclusion have enabled the plant to navigate and avoid more salt and its associated toxicity problem and at the same time, compensating such action by adjusting K^+ uptake, which has been shown to improve osmotic adjustment and enhance cell turgor (Munns and Tester, 2008).

1.3.7.3 Tissue Phase

Tissue tolerance is a term used to describe the plant's ability to grow under salt conditions (Munns and Tester, 2008). The tissue tolerance mechanism is about ensuring the survival of old leaves and improving growth (Munns and Tester, 2008). The compartmentalisation

of Na⁺ and Cl⁻ ions at the cellular and intracellular level is requisite in tissue tolerance phase in order to prevent the toxicity effects of higher concentrations of these ions to the mesophyll cells in the leaf (Munns and Tester, 2008). Also significant is an increase in accumulation of compatible solutes within the cytoplasm under salt stress. The compatible solutes are examples of small molecules, water soluble, derivatives of sugars, phenolic amino acids, and other complex sugars (Ashraf and Foolad, 2007). They play an important role in osmo-tolerance, protecting enzymes from denaturation, thereby ensuring membrane and macromolecules stability mediating the osmotic adjustment (Ashraf and Foolad, 2007). Their main functions are not limited to balancing and osmotic adjustment as they are involved in regulation of hydrophilicity, and replacement of water molecules at the surface of proteins and membranes, by creating and serving as low molecular weight chaperones (Hasegawa et al., 2000). Studies have also reported their involvement in a scavenging function against the ROS in the stabilisation of cellular structures (Hasegawa et al., 2000; Zhu et al., 2015). Their unique characteristics have attributed to their neutral nature, therefore high accumulation of compatible solutes do not affect cellular processes (Sakamoto and Murata, 2002). Examples of the known compatible solutes include; proline and glycine betaine (GB) levels of both have been shown to increase significantly under salt and drought stresses (Munns, 2002; Sakamoto and Murata, 2002). They constituted a significant amount of the metabolites reported to be increased in studies involving durum wheat under salt stress (Sairam and Tyagi, 2004; Ashraf and Foolad, 2007). Halophytes have also been reported to have an improved osmotic pressure, which was attributed to the increasing accumulation of compatible solutes such as proline and GB (Flowers et al., 1977). In the glycophytes, studies have shown the concentrations of the compatible solutes are lower compared to halophytes. Durum wheat has been shown to have improved osmotic adjustment through a high proline content (39%), on the other hand, GB has shown to

contribute up to 16% of the osmotic balance in both cytoplasmic compartments of both older and younger leaves tissues (Carillo et al., 2008).

1.3.8 Molecular Basis of Regulatory Networks in Plant Salt Stress Signalling Pathways

The plant's genetic response to salt stress is mechanistically complicated and has been shown to involve strict regulation of significant players, e.g., proteins and RNAs. The mechanism has evolved to act at different stages of regulation that could be described by central dogma, where transcription leads to mRNA synthesis followed by post-translational modification, translation, and post-translational modification where a protein is coded and decoded (Gupta and Huang, 2014). Knowledge of the regulation of gene expression at the mRNA level has provided insight on how plants behave in response to extreme changes due to environmental cues (Gupta and Huang, 2014). Proper profiling of transcriptional processes has been widely used to screen out the candidate genes involved in abiotic stress responses. Information on salt-responsive genes, transcription factors, up-regulation or down-regulation have been documented using microarray data and transcriptomic profiling (Johnson et al., 2006). Furthermore, advances in molecular biology techniques and approaches such as functional genomics have contributed significantly towards identification, cloning, and characterisation of these genes (Johnson et al., 2006). The role of transcription factors in gene expression under salt conditions have been reported and the interaction has been considered to be the most crucial in up- and down-regulation of key genes, which could determine the plant's adaptability to a salt environment (Johnson et al., 2006). For example, zinc finger genes; *bZIP*, *WRKY*, *AP2*, *NAC*, *C2H2*, and MYB and DREB family proteins have been considered to be the most stress-responsive members (**Figure. 1.6**) in abiotic stress signalling pathways. These transcription factors have shown

to have the capacity to alter gene expression by *Cis-acting* specific binding in the promoter region of many genes (Johnson et al., 2006).

Moreover, a quite significant number of drought/salt stress inducible-genes have been discovered through microarray analysis of *Arabidopsis* transcriptome and other grasses, such as rice (*Oryza sativa*) (Takasaki et al., 2010). They have been reported to play vital roles in plants stress tolerance and have the potential to coordinate genes expression through signal transduction in many plant cells (Xiong et al., 2003; Takasaki et al., 2010). Efforts have been undertaken to unmasked the molecular mechanisms involved in the regulation and control of gene expression in response to abiotic stresses (Takasaki et al., 2010). The initial studies have been reported and aimed to establish the key role of *cis*- and *acting* elements and how they affect modulation of the stress response by using model dicot plant *Arabidopsis thaliana*; and some studies involving rice (*Oryza sativa*) (Egawa et al., 2006; Agarwal et al., 2007).

The transcription factors have shown to bind to the *cis-acting* elements, these are elements involved in transcription of stress-gene in the promoter region of a target genes thereby affecting its expression, and the complete process of transcriptional regulations is known as the regulon. Several such regulons are actively involved in response to abiotic stress have been identified using the model plant *Arabidopsis thaliana* (Nakashima et al., 2009).

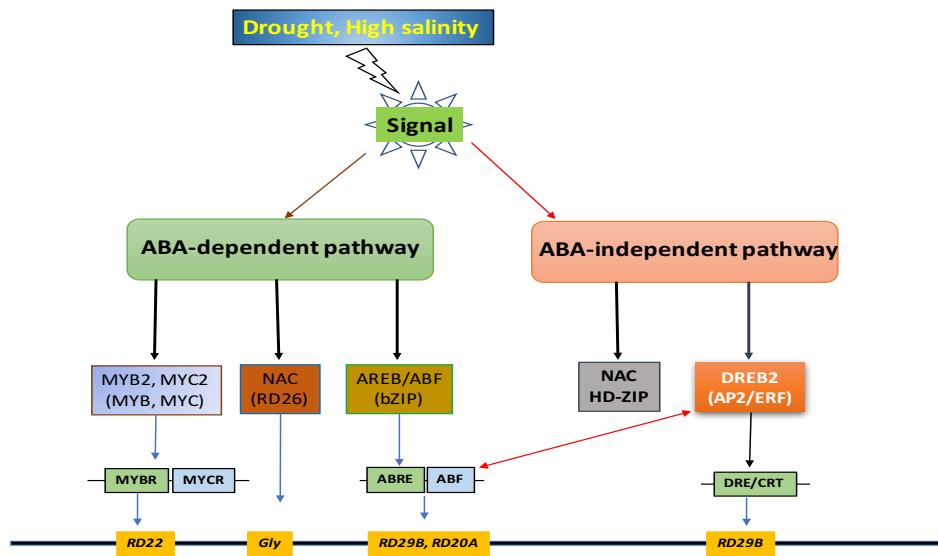


Figure 1.6: Schematic diagram of the plant regulatory networks induced by drought/salinity stress, which involves the activation of both pathways of Absciscic acid-dependent (ABA-dependent) and Absciscic acid independent pathways. This involves the binding of transcription factors (TFs), such as MYB2, MYC2, NAC, AREB/ABF, NAC HD-ZIP and DREB2 to specific cis-acting elements (MYBR, MYCR, ABRE/ABF and DRE/CRT) which leads to the expression of drought/salinity stress-responsive gene such as: RD22, Gly, RD29B, and RD20A respectively (Nakashima et al., 2009).

The transcriptional regulatory networks established by using a model of signalling cascade pathways have shown to comprised two critical operational paths, that have shown to become activated in response to either abiotic/biotic stresses. These have been referred to as ABA-dependent and ABA-independent signalling pathways respectively. This pathway regulates signal transduction and eventual expression of elements responsive to genes including *RD29B* and *RD20A* genes. MYB2 and MYC2, are also involved through ABA-dependent stress-inducible genes that have shown to influence the appearance of the *RD22* genes. Additionally, MYC2 has also been shown to be involved in the metabolic pathway related to JA-inducible genes that are triggered following most biotic stress's especially those that are pathogen related. Other important transcription factors that have shown to play vital roles include, RD26 NACs, which operate through dual pathways; ABA-dependent and JA-inducible gene expression, in response to stresses (Nakashima *et al.*,

2009). The cross-talking between abiotic and biotic stress responses involves activities of MYC2 and NAC transcription factors. In the ABA-independent pathway, DREB2s are the main transcription factors; and are activated in response to dehydration and high salt stresses. Furthermore, dehydration response elements (DREs) have been shown to become operational via cis-acting elements operating through the same pathway to modulate and regulate genes for drought and salt stress. (Nakashima *et al.*, 2009).

1.3.9 Mapping Genes Concerning Plant Salt Tolerance

Polygenes that control many agronomic traits of important such as yield, quality and other forms of disease resistance have been reported (Asins, 2002). The principal method of identification of genomic region that contributes to a phenotypic variation is referred to as quantitative trait loci (QTLs) mapping. QTLs have been typically linked to or shown to contain genes that have been invariably involved in controlling observed phenotypic variation (Mikiko *et al.*, 2001). Fragments of DNAs identified as genetic markers can be used to identify particular points within the genome that represents genetic differences (Asins, 2002).

Genetic markers have been categorised based on their technical requirements; those genetic markers that have shown to detect the whole chromosomes, and those that have a specific (fragments) of genetic variation have been reported (Mikiko *et al.*, 2001). Among the earliest type of DNA markers discovered were Restriction Fragment Length Polymorphisms (RFLPs), which were shown to detect variation in restriction fragment size using southern hybridization. This technique, enables the detection and or removal of a restriction endonuclease recognition site, which resulted from a single nucleotide base alteration which caused a shift in fragment length (Horst and Wenzel, 2007). RFLP markers remain valuable tools that are used in breeding programs, however, advances and

development of simple single repeat (SSR) markers superseded the use of RFLP markers. SSR markers allow detection of variation in the number of short repeat sequences, they could be of two or three base pairs that are repeated in multiple occurring sequences in the genome of a species (Horst and Wenzel, 2007).

Other reported DNA markers that have been developed are the expressed sequence tag (EST) databases and single nucleotide polymorphisms (SNPs) that occur at varying frequencies in the different regions of the genome of any given species (Horst and Wenzel, 2007).

1.3.9.1 Mapped Genes Related to vacuolar (*NHX1*) and plasma membrane (*SOS1*) Antiporter Genes Associated to Plant Salt Stress Tolerance

Ion exclusion is one of the essential mechanisms involved in salt stress tolerance. Factors that have been reported to enhance low cytoplasmic Na^+ concentrations in plant cells have been shown to be associated with the tonoplast Na^+/H^+ exchanger 1 (*NHX1*) (Yamaguchi et al., 2013) (**Figure 1.5**). The activity of the plasma membrane-bound protein SALT OVERLY SENSITIVE (**Figure 1.5**) (*NHX7* in *Arabidopsis*) Na^+/H^+ antiporters have also been implicated in Na^+ ion exclusion (Blumwald and Poole, 1987; Qui et al., 2002; Yamaguchi et al., 2013). The role critically played by *NHXs* gene has been primarily shown to do with Na^+ exclusion through sequestration of Na^+ within the vacuole (detoxification process), while the SOS pathway has been shown to be involved in the exporting Na^+ ions out of the cell. Additionally, overexpression of *AtNHX1* and its analogues in *Arabidopsis* and other plant species, such as tomato (*Solanum lycopersicum*), and rice have been reported where they have been shown to improved salinity tolerance (Apse et al., 1999; Zhang and Blumwald, 2001). Recent studies have demonstrated other roles played by *NHX*-type

proteins in K^+ compartmentalisation into the vacuole for cellular pH homeostasis and maintenance (Barragan et al., 2012). Furthermore, overexpression of *AtNHX1* in tomato has been shown to improved vacuolar K^+ and its transport from the root into the shoot (Leidi et al., 2010; Bassil et al., 2011; Barragan 2012).

A successful QTL mapping of increased leaf Na^+ accumulation in tomato has identified *LeNHX3* (Villalta et al., 2007). This demonstrates that NHX antiporters have more than one role in osmoregulation, cell growth, and plant development (Bassil et al., 2011; Barragan et al., 2012). Other studies have suggested an involvement of endosomal transport proteins, including *NHXs* genes in plant salt tolerance that show its involvement in pH regulation, especially in the subcellular organelles and ion homeostasis (Krebs et al., 2010; Bassil et al., 2011; Yamaguchi et al., 2013). In addition, two isoforms of *NHXs*, *NHX5*, and *NHX6*, have been reported that are localised in Golgi and *trans*-Golgi networks. *nhx5 nhx6* double knockout plants have been shown to demonstrate hypersensitivity to salt stress (Leidi et al., 2010). Likewise, studies involving loss of function have shown that vacuolar transporter H^+ -ATPase in a mutated *Arabidopsis* have been attributed to unimpaired salt tolerance, i.e., not significantly shown to alter the salinity stress in *Arabidopsis*. Other studies, however, have reported the reduction of V-ATPase activity in the *trans*-Golgi network/early endosome (TGN/EE), which resulted in an increase salt sensitivity (Villalta et al., 2007). Overexpression studies involving vacuolar-type I H^+ -PPase AVP1 have also been shown to improve plant salt tolerance via vacuolar Na^+ sequestration (Undurraga et al., 2012). Furthermore, overexpression of *AtAVP1* in crop barley (*Hordeum vulgare*) have implicated H^+ -PPase in the improvement of salinity tolerance under greenhouse controlled environment by substantially improving shoot biomass and grain yield (Schilling et al., 2013).

1.3.9.2 Overexpression of High Potassium Membrane Transporter Genes (*HKT*) and Plant Salt Stress Tolerance

In order to find the role of HKT in salt tolerance, several studies have been carried out. These have involved identification of the role of *HKT1* in wheat (*Triticum aestivum*) using gene (*TaHKT2;1*), which was shown to mediate in Na^+/K^+ cation transport (Schachtman and Schroeder, 1994; Rubio et al., 1995). Further studies in the characterization of different isoforms of *HKT* genes that have been found present in different plants species have been reported (Horie et al., 2007). Different methods used in the analysis and characterisation of HKT have shown the presence of other copies of HKT transporters isoforms (Uozumi et al., 2000; Mäser et al., 2002). Subsequently, two distinct subgroups have been identified; class I and II, which are believed to mediate Na^+ -selective transportation (Uozumi et al., 2000; Mäser et al., 2002), and are involved in co-transportation of Na^+-K^+ (Rubio et al., 1995). Studies have reported that mutated *HKT* gene in *Arabidopsis*, *AtHKT1;1*, encodes for a class I transporter, which has shown to causes high Na^+ sensitivity in the mutants (Mäser et al., 2002; Horie et al., 2007). Some detail analyses using mutant have indicated that *AtHKT1;1*, and its orthologues in rice, *OsHKT1;5*, play a critical role in the efflux of Na^+ from the xylem sap into the surrounding xylem parenchyma cells. Thereby, enhancing the protection of leaf from Na^+ toxicity (Ren et al., 2005; Sunarpi et al., 2005; Horie et al., 2006; Davenport et al., 2007). An overexpression of *AtHKT1;1* in a target stele has shown to improves salt tolerance (Moller et al., 2009). Studies involving *in vivo* electrophysiological analyses using stellar cells from wild-type and *Athkt1;1* mutant plants have shown to demonstrate the role played by *AtHKT1;1* mediating Na^+ transport through channel-like transporters (Xue et al., 2013; Xue et al., 2016). It has been suggested that *AtHKT1;1* gene play a role in facilitating Na^+ removal from the xylem and indirectly stimulating K^+ loading into xylem vessels. The

K⁺ filling was supposed to be carried out through outward rectifying K⁺ channels, leading to the improvement of K⁺/Na⁺ ratio in the leaves (Ren et al., 2005; Sunarpi et al., 2005).

QTL analyses on Na⁺ resistance have indicated that xylem Na⁺ unloading mechanisms have been essential for salt tolerance on both rice and wheat (*Triticum turgidum*) (James et al., 2006). Salt tolerance QTLs have been linked to the regions containing to *HKT1;5* orthologues, which encodes for increased Na⁺ - exclusion and in particular for a class I HKT transporter (Ren et al., 2005; James et al., 2006; Byrt et al., 2017). Another QTL linked to Na⁺ tolerance has been correlated with a critical role played by *the Nax1* gene for salt tolerance (Munns et al., 2012). Further work on *Nax1 locus*, have been linked to the region of the *TaHKT1;4*, gene that encodes for a class I HKT transporter (Huang et al., 2006).

HKT marker-assisted studies have shown to record some successes through the introduction of a wheat *HKT1;5* from an old wheat relative *Triticum monococcum* into commercial durum wheat (*T. turgidum* ssp. *durum* var. Tamaroi). Studies on transgenic varieties of this gene have shown to improve tolerance to salt stress (Munns et al., 2012). Finally, it has been suggested that the maintenance of K⁺ acquisition with the exclusion of Na⁺ from leaves have a direct correlation with plant salt tolerance (Hauser and Horie, 2010).

1.3.10 Salinity Tolerance among the *Brassicas*

Besides using *Arabidopsis* as a model plant, the second most widely used plant model system of the agronomic (traits) values are the *Brassicas*. This is because they are rich in genetic diversity and significant inter and intraspecific variation for salt tolerance has been identified which could be exploited through selection and breeding programs for salt tolerance enhancement. The stages of seed germination and early seedling growth have been demonstrated to be relatively sensitive to salinity (Ashraf and McNeilly, 2004).

1.4 Breeding for Salt Tolerance in *Brassicas*

1.4.1 Conservation of *Brassica* Germplasm for Breeding Programme

In a search for reliable and conserved sources of variability the gene banks are relied on to regulate the loss of genetic variability in the domesticated gene pool. This has become more common through the setting up of many genetic diversity centres across the regions of the world. A collection of *Brassica* wild germplasm was first reported in the 1970s and research using cytogenetic studies have led to the conservation of many useful germplasm and landraces, which could have been lost due to genetic drift (Gómez-Campo *et al.*, 2006, 2007). Following these successes in the 70s, the *Brassica* germplasm has been boosted by setting more centres for biodiversity for the *ex-situ* and *in-situ* collections. Two types of gene banks have been set-up: **Seed bank**, and **Gene bank**, each of which has shown to be complementary from one another and have been used to represent the conservation processes of both *in-situ* and *ex-situ*. The Seed Bank, has been more *in-situ* oriented, and designed to carry out three main significant responsibilities; 1) ensures a good record of seeds collected from different diversity of genetic *Brassica* crops, 2) ensures good storage conditions to avoid genetic erosion, and 3) ensures supply for research purposes (Gómez-

Campo 1999a). The Gene Bank, on the other hand, has two significant roles; 1) collection of traditional varieties and landraces around the globe, and 2) centres of genetic diversity of specific crops using accessions for research and regenerations purposes (Gómez-Campo 1999a).

The most extensive seed bank for *Brassica* collection centre established has been that of the Universidad Politécnica of Madrid (UPM), Spain in 1966. This has a broad aim for both long-term *ex-situ* conservation and ensures availability of different accessions for research. It has been reported to have an initial collection of over 600 plants (accessions) of crucifer in the Plant Germplasm Bank and has also had a public collection around the western Mediterranean coast (Gómez-Campo 1999a). Other collaborative efforts across Europe have also been reported such as the International Board for Plant Genetic Resources (IBPGR), International Plant Genetic Research Institute (IPGRI) (Gómez-Campo and Gustafsson 1991). The centre has been transformed to a global centre of biodiversity representing different regions, for examples, coastal regions of Spain, Italy, Greece and Tunisia and the Atlantic coast of Spain, France, and the UK. Thereby ensuring large collection of wild *B. oleracea* species ($n = 9$) and its related species; *B. macrocarpa*, *B. rupestris*, *B. incana* and *B. villosa* have been collected around Sicily (Gómez-Campo and Gustafsson 1991).

1.4.2 Screening of Salt Tolerance from Pre-existing genotypes of *Brassicas*

Salt tolerance is a complex trait and has been shown to be controlled by cross-talk mechanisms from the cell to the whole plant (Greenway and Munns, 1980). Screening of secondary gene pools comprising the amphidiploid (allotetrapolyploids) species; *B. carinata*, *B. juncea*, and *B. napus*, indicated superiority in salt tolerance compared to the

diploids; *B. campestris* (syn: *rapa*), *B. nigra*, and *B. oleracea* (Makela et al., 1999). The complexity of the salt traits has shown to be different among Brassica species. Because of its quantitative nature, the effect of one mechanism may exclude the significance of the others at certain physiological stages of plant development (Greenway and Munns, 1980; Gorham et al., 1991; Ashraf, 1994; Makela et al., 1999). The cultivated *Brassica* species comprising both diploid and amphidiploid (allotetrapolyploids) species and other wild *Brassica* species are considered to be moderately salt tolerant (as mentioned in Section 1.1.2 and 1.1.5), because of their natural growing environment. Some reported studies have favoured the amphidiploid species, *B. carinata*, *B. juncea*, and *B. napus* over the diploid species, *B. campestris*, *B. nigra*, and *B. oleracea* (Malik, 1990; He and Cramer, 1992; Kumar, 1995). Ashraf et al., (2001) have further demonstrated that the salt tolerance of amphidiploids (allotetrapolyploids) was acquired from both the diploid genomes (A & C), i.e., from *B. campestris* and *B. oleracea* species. A study by Purty et al. (2008) on screening for salinity tolerance observed significant interplay and specific variation within the *Brassica* genera. Such differences have also been observed in responses to growth and physiochemical parameters. For example, electrolyte leakage, proline accumulation and the K^+/Na^+ ratio have shown to be of genotype depended (Ashraf and McNeilly 2004).

1.4.3 Conventional Breeding Approaches for Developing Salt Tolerance in *Brassica*

Screening of salt tolerance using traditional breeding involving crop varieties has proven to be promising. The economic cost of soil reclaiming once it has become saline is beyond what an ordinary farmer can afford (Ashraf and McNeilly, 2004). A gene pool that has shown to be rich with a heritable variation that can to provide the inherent variability needed has become the prerequisite for any successful breeding program. Good parental genetic

diversity serves as a good source of starting material such as well-adapted landraces that can be used to enhanced local adaptation (Ashraf and McNeilly, 2004). It can also be used to overcome the problem associated with soil salt susceptibilities and thereby provide the needed foundation for breeding requirements (Ashraf and McNeilly, 2004).

In the developing of a salt tolerant plant, the use of conventional breeding approaches has been yielding progress. Its applications have been demonstrated in the generation of a different variety of salt-tolerant *Brassicas*. Studies reported by Central Soil Salinity Research Institute, Karnal, India, have shown to be quite promising and led to the development of more salt tolerant varieties of *B. juncea* cultivars; CS52, CS54, CS416, CSTR 330-1, CSTR 600-B-10, CSTR 610-10-1-1, and CS12 respectively. The work further showed *B. juncea* var. CS52 cultivar tested to grow in the saline land (having EC 7 to 8 dsm-1 and pH 9.2 - 9.3) with an average good yield (Sadiq et al., 2002). Comparative performance among the various varieties of *Brassica* cultivars has shown that cultivar P-8-2 to be more salt tolerant as compared to Peela raya, SPS-23-1, SPS-23-2, ORI-56-6 cultivars (Sadiq et al., 2002).

1.4.4 Genetic Engineering (Transgenic) Approaches for Salt Tolerance Enhancement

The advances in molecular genetics and genetic engineering have provided essential tools for the study of salt tolerance. Tools such as transgenic approaches enable us to address the problem of salt stress. It has been shown how these can impact on the development of enhanced salt-tolerant plants. Although no fully salt-tolerant plant has been obtained by genetic engineering, the use of multiple tolerance mechanisms for one or more of the abiotic stresses through stepwise or co-transformation have shown to be helpful in the quest for high-level salt-tolerant plants for commercial purposes (Bohnert and Jensen, 1996).

Additionally, Bohnert and Jensen (1996) have suggested that the generation of salt-tolerant plants through metabolic engineering would involve the massive transfer of a different set of genes to the affected plant. Advances in biomolecular techniques nowadays have made the idea more conceivable and credible, such techniques have shown to provide channel for identification of specific genes that could respond positively and negatively through combination of RNA and Proteins analyses with a particular focuses on transforming the plants (Kawasaki *et al.*, 2001; Salekdeh *et al.*, 2002).

1.4.5 Formation of Diversity Foundation Sets

1.4.5.1 *B. oleracea* Genetic Diversity Foundation Set (Bol DFS)

The potential of the *B. oleracea* C-genome (BolCg), gene pool could be exploited to serve as the reservoir of favourable alleles that could be useful to plants breeding programme primarily in search of salt-tolerant *B. oleracea*. The advancement of biological techniques, introgression of relevant alleles for the improvement of cultivated *B. oleracea* vegetables could be facilitated.

The BolDFS are genetically diversity of wild *B. oleracea* accession lines, some of the names have been earlier mentioned (Section 1.1.6), which are not genetically fixed but have come from a collection of over 89 accessions representing 14 species (see Table S1 Appendix I), which further represent within 386 founder accessions gene pool based on the EU GENRES core collection (Leckie *et al.*, 1996) and their relation to DFFs production is being outlined in **Figure 1.7**.

1.4.5.2 Formation of *B. oleracea* C-genome Genetic Diversity Fixed Foundation Sets (BolCg DFFSs)

The production of DFFSs (S1) lines (**Figure 1.7**) for *B. oleracea* was initiated to capture the diversity of *B. oleracea* and other brassica materials held at Horticulture Research International (HRI, Wellesbourne, UK) (see Table S2 Appendix I). These were based on valuable genetic and genomic resources of *B. oleracea* and related wild C-genome *B. oleracea* species (BolDFSs) (Babula *et al.*, 2007; Walley *et al.*, 2012). The concept of core collections has been developed to further generate homozygous doubled haploid (DH) lines from microspore culture (Pink *et al.*, 2008; Walley *et al.*, 2012).

Both BolDFS and S1s have been shown to represent diversity within the gene pool and significant effort have been made to fix the S1s through selfed (**Figure 1.7**) after many generations from the original founder wild accessions and are considered to be 50% more homozygous as compared to the founder wild accessions BolDFS lines (Pink *et al.*, 2008; Walley *et al.*, 2012).

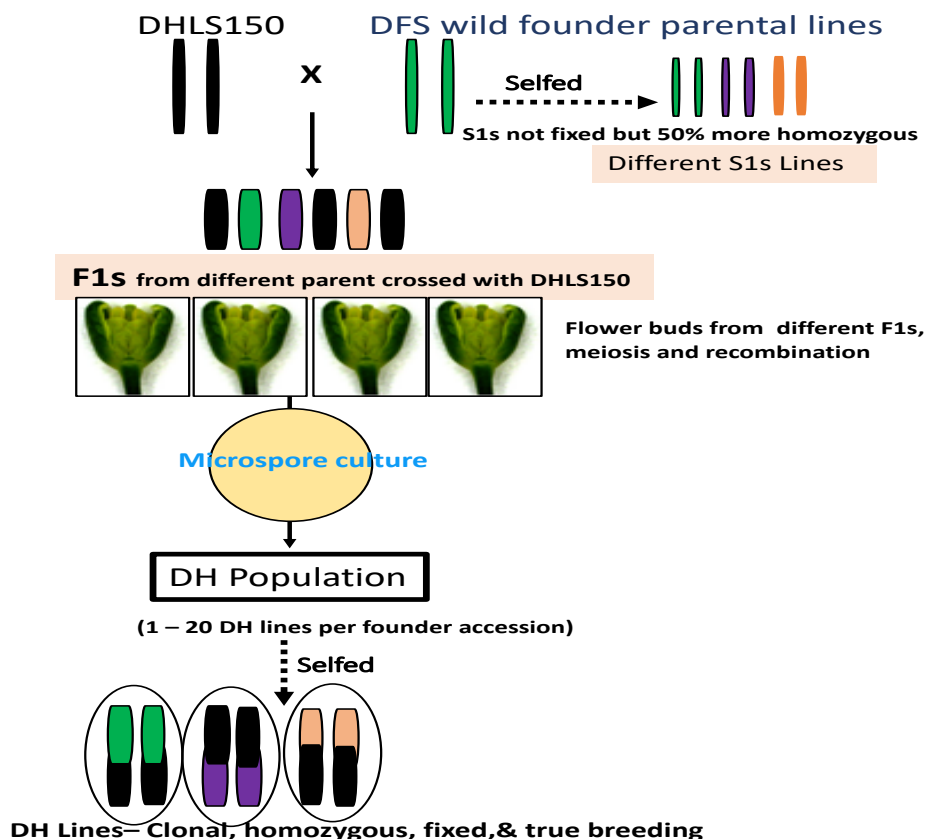


Figure 1.7: An outline presentation describing the relationship between the **BolDFSs**, **S1s** and **DH** population, stages involve and material used. The **BolDFSs** are main founder parent lines for **S1s** (50% homozygous) following generations of selfed, the **DHSL150** is a doubled haploid line, genetically fixed (homozygous) (see Section 1.3.5.3) for its description and formation. The **F1s**-hybrids materials obtained were sown and flower buds collected and microspore tissue culture was used and an anti-microtubule agent colchicine used for chromosome doubling for **DH** populations and 1 – 20 **DH** lines per accession line was obtained.

1.4.5.3 Generation of homozygous Doubled Haploid (DH) Lines of *B. oleracea* C-genome Diversity Fixed Foundation Sets (BolCg DFFSs)

The concept of core collections has been developed to further generate homozygous doubled haploid (DH) lines from microspore culture (Pink *et al.*, 2008; Walley *et al.*, 2012). These are clonal, true breeding lines that were genetically fixed. They are obtained from the F1-hybrids from the crosses between the **DHLS150** (**Figure 1.7**), which is rapid cycling, self-compatible and microspore responsive and by using different wild founder accessions of **BolDFS** lines, which are not genetically fixed, self-incompatible and not microspore responsive, these are due to strong effects of S-alleles in their S-locus (Pink *et al.*, 2008;

Walley *et al.*, 2012). Other characteristics of wild founder BolDFS include; longer time to produce flower and poor seed quality. The diagrammatical presentation for stages and materials used in the production of DHSL150 are outlined in **Figure 1.8** respectively.

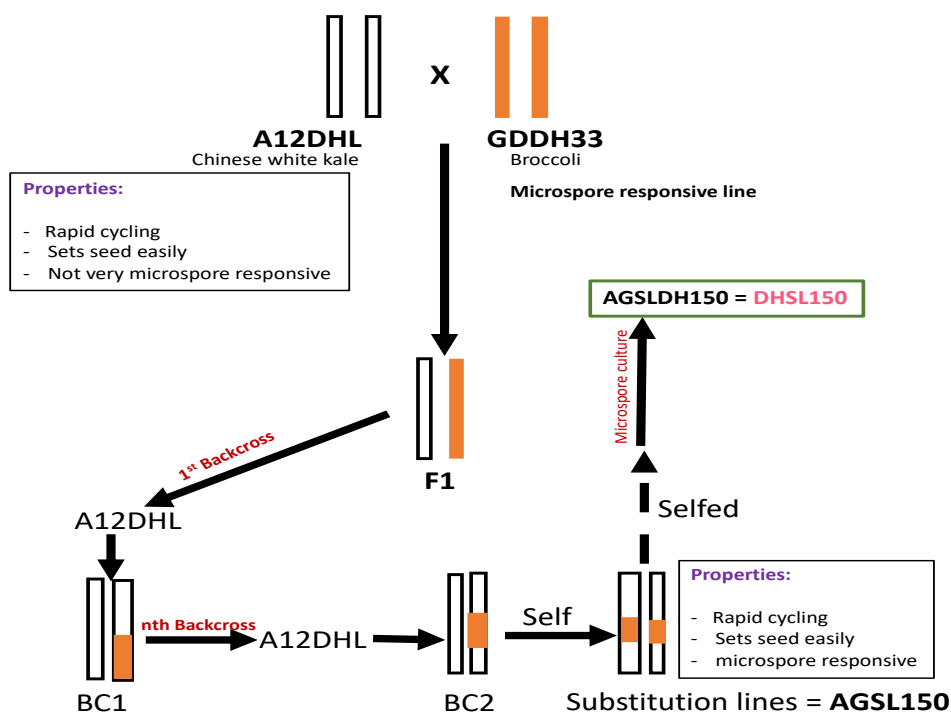


Figure 1.8: DHSL150 was produced using two parent materials: **A12DHL** (Chinese white kale), a DH line, rapid cycling, sets seed easily but not microspore responsive crossed with second parent **GDDH33** (Broccoli), a DH line, microspore responsive. The **F1s**-hybrids obtained are backcross to A12DHL for generations to obtain the substitution lines **AGSL150**, which undergo selfed stages and finally, flower buds collected and microspore culture used to generate the **DHSL150**.

1.5 High throughput Molecular Biology Techniques

The development of high throughput technologies in recent times have helped to achieved mapping and quantification, which are highly efficient methods that enable simultaneous detection and measurement of genes expression for a given cell (Tuteja and Tuteja, 2004). Generally, methods used for assaying gene expression are grouped into two; 1) closed method, which requires prior information about the gene to be measured or any collected clones or sequences, and 2) open method, which requires no such prior knowledge before genes being analysed (Tuteja and Tuteja, 2004). Furthermore, techniques that are based on subtractive hybridisation and differential display such as microarray-based methods and Sanger sequencing or Expressed Tag Sequencing (EST) libraries have been employed and used, however, these approaches have application limitation due low throughput, cost and nonquantitative nature (Douglas *et al.*, 1999; Gerhard *et al.*, 2004). To overcome these challenges, throughput techniques were developed e.g., serial analysis of gene expression (SAGE), cap analysis of gene expression (CAGE), and massively parallel signature sequencing (MPSS) have been developed and currently in use (Velculescu *et al.*, 1995; Brenner *et al.*, 2000; Reinertz *et al.*, 2002; Shiraki *et al.*, 2003; 2005; Kodzius *et al.*, 2006; Peiffer *et al.*, 2008). These sequencing techniques are high throughput give precise digital gene expression quantification analysis.

1.5.1 Serial Analysis of Gene Expression (SAGE)

SAGE, this is a high-throughput method with good efficiency for robust detection and measurement of expressional levels of genes in a given cell. The method is mainly work based on two basic principles; a short oligonucleotide sequence tag (10 – 11 base pairs) that contains information to uniquely identify a transcript. These tags are being used to identify genes and their transcripts relative abundance in mRNA, the second principle is by concatenating the short sequence tags this will enable efficient analysis of transcripts in a

serial manner since SAGE uses serial processing (Renu and Tutaja, 2004). The method involves key stages such as; generation of short nucleotide sequences (tags). These are made from a unique position within each species of mRNA, conversion of the mRNA to cDNA by using biotinylated double-stranded cDNA, this follows with digestion using an anchoring enzyme, isolation, and linkers to linkage, amplification of tag-linkers (PCR) and finally, constructs digestion, ligated to concatemers, clone and sequenced. This allows rapid sequencing, quantification, and identification of cellular transcripts (Velculescu et al., 2000).

1.5.2 Massively Parallel Signature Sequencing (MPSS)

MPSS has been developed as the newest tools to conduct an in-depth gene expressional profiling. It uses an open-ended platform in the analysis of virtually all genes present in individual mRNA molecules in a given sample and produced each gene. The method needs no prior knowledge about genes to be identified and characterised before the experiment. It has high sensitivity compared to other hybridisation based methods, microarray and non-microarray techniques (Zhou et al., 2000). Its digitalised databases for handling data processes and bioinformatics tools have simplified and added to its advantage for data management and analysis, where genes analysis and sorting out the number of mRNAs from each gene relative to the total number of molecules in a given sample can be performed simultaneously. It offers more ways for generating complete datasets that will help to facilitate hypothesis-driven molecular experiment (Jeannette et al., 2001). When it comes to the generation of complete datasets for building relational databases, MPSS has edged microarray platforms and SAGE (Zhang et al., 1997; Duggan et al., 1999).

1.5.3 Plants Molecular Genotyping: Genotype-by-Sequencing (GBS)

Genotyping-by-sequencing (GBS) has become feasible as a result of advances in next-generation sequencing (NGS) that have driven down the cost of sequencing and ultimately made GBS accessible as a tool (Elshire et al., 2011). GBS has become a simple multiplexed system that enables construction of a reduced representation of libraries which can be sequenced on the Illumina NGS platform (Elshire et al., 2011). GBS detects large numbers of SNPs that can be used in genetic analyses and genotyping (Beissinger et al., 2013). GBS requires less sample handling, fewer PCR and purification steps, no size fractionation and reference sequence limits, and finally efficient barcoding (Davey et al., 2011). Different library protocols of GBS have been developed and described by Sonah et al. (2013). Different GBS strategies can be used including the use of Ion PGM system and by using A) Restriction enzyme digestion and B) Multiplex enrichment PCR (Poland et al., 2012a).

The use of sequencing restriction site associated genomic DNA (RAD) has proved valuable for high SNPs discovery and genotyping as reported by Baird et al. (2008). Increased accessibility through cost benefits and efficiency by incorporating a multiplex sequencing that uses a barcode system has been the turning-point for GBS. The advantage of barcodes that are used in one of the adapter sequences, recognised by restriction enzyme (RE) upstream cut-site in genomic DNA eliminates the need of second Illumina sequencing read (Baird et al., 2008). Comparing the two approaches i.e., RAD method and GBS, genotyping-by-sequencing has been substantially less complicated, straightforward, single-well digestion of genomic DNA etc. outlined in **Figure 1.9** has made GBS more attractive and apply in many application (He et al., 2014).

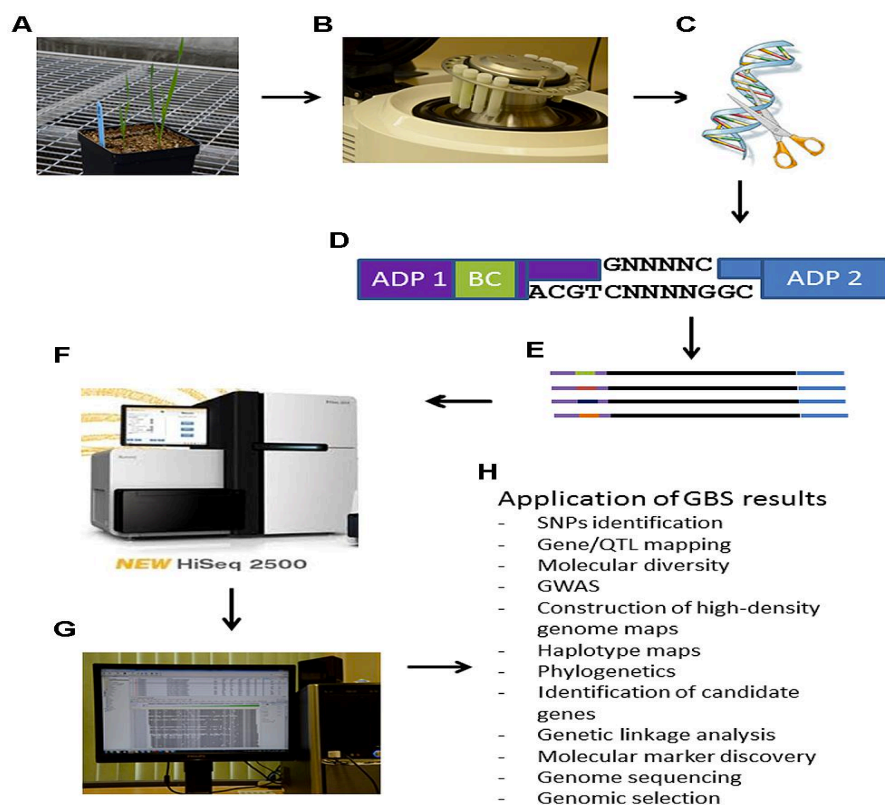


Figure 1.9: Diagram showing the stages involved in carrying out genotyping-by-sequencing (GBS) for plant breeding. **(A):** plant tissue **(B):** ground leaf tissues for DNA isolation, quantification, and normalization. At this step it is important to prevent any cross-contamination among samples; **(C):** DNA digestion with restriction enzymes; **(D):** ligations of adaptors (ADP) including a barcoding (BC) region in adapter 1 in random restricted DNA fragments; **(E):** representation of different amplified DNA fragments with different barcodes from different biological samples/lines. These fragments represent the GSB library; **(F):** analysis of sequences from the library on an NGS sequencer; **(G):** bioinformatic analysis of NGS sequencing data; **(H):** possible application of GBS results. (He et al., 2014).

1.5.5 An Outline of Molecular hybridisation techniques for qPCR

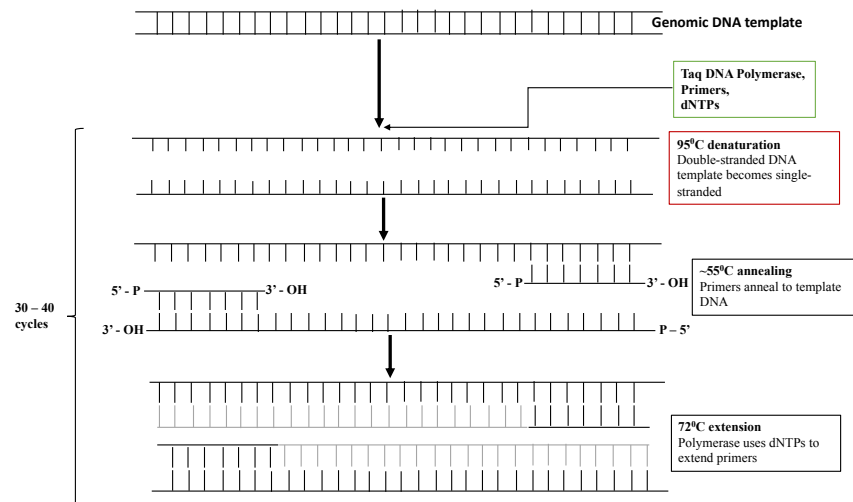


Figure 1.10: The polymerase chain reaction (PCR). Specific sequences of DNA are amplified by PCR using pairs of oligonucleotide primers. Genomic DNA, primers, Taq DNA polymerase, and deoxynucleotides (dNTPs) are combined in a suitable buffer and subjected to repeated cycles of alternating temperatures which denature the template DNA, allow for primer annealing to the template and finally extend the primer to create a new strand DNA (Tagu and Moussard 2003).

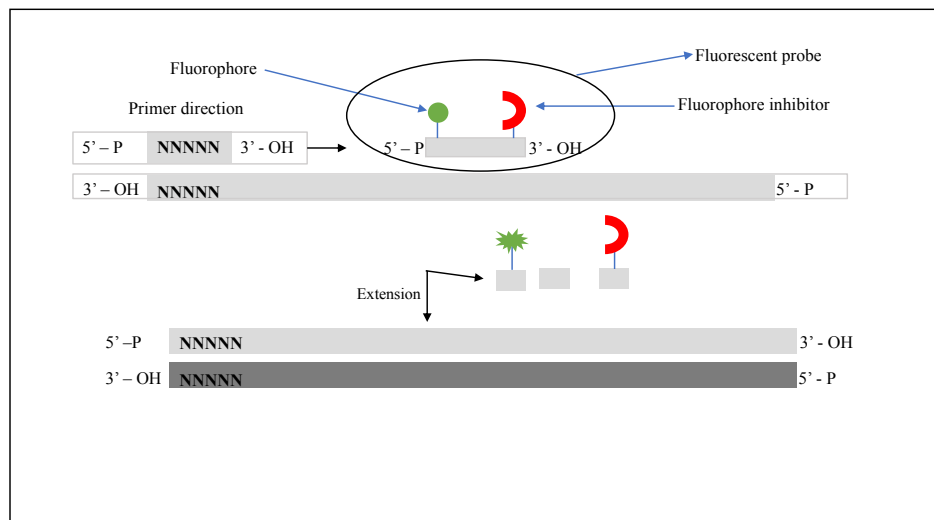


Figure 1.11: Real-time PCR using hydrolysis (TaqMan) probes. PCR primers are used to amplify target DNA as in standard PCR. During the annealing step of each PCR cycle a fluorescently labelled oligonucleotide probe, complementary to one strand of the template DNA, anneals along with the primers. The TaqMan probe contains one detection dye (shown in red) whose fluorescence is absorbed by another quencher dye (shown in green) when a probe is intact. During an extension step of each PCR cycle, the TaqMan probes are digested. This physical separation of the dye from the quencher allows for increased fluorescence during the extension step of each PCR cycle (Tagu and Moussard 2003).

1.6 Screening the *B. oleracea* Breeding Lines (DFFSs) for Salt Stress Resilience

1.6.1 Initial Salt Stress Screening leading to Line Selection for main Thesis.

52 brassica oleracea DFFS lines, (only the S1s), selfed from the original wild founder lines as described in Section 1.3.5.2 and Figure 1.3 were selected based on availability of seed. They were comprised of different *Brassica oleracea* species as listed in Table SII in Appendix I. In 2014 between May – September, a salt stress experiment was designed in conjunction with Dr Peter Walley. From the design, six replicates of each line (three control and three treated plants) were paired and randomly distributed. Seeds from the 52 lines selected were provided by G. Teakle and the GenBank sourced from the DEFRA funded VeGIN project. The initial experiment setup was executed by Christine Hicks assisted by Gary Grant, in the glasshouse Phytobiology unit. The plants were grown for 4 weeks in smaller plastic pots before being transplanted into bigger pots. LD50 was determined using some spare plants that were not included before the main experiment. Different concentrations of salt (NaCl) were prepared, 350 mM, 300 mM, and 250 mM respectively. Some plants died between 350 mM – 300 mM and the idea was not to kill the plants but observed some immediate salt stress response and by following the Systems biology approach to interpreting the data, therefore 250 mM salt concentration was adapted and mode of salt administration to be as shock (single dose) by feeding the treated lines with 250 mM NaCl.

In addition, plants were treated at week six (6), at least each with four completely expanded leaves. They were allowed to grow for three additional weeks. My role in this experiment started with the data collection and measuring morphological traits; plants dry weight, leaf dry weight, where plants height and leaf area were measured by using photographs of respective lines and leaves in ImageJ respectively. Based on data collected and analysed

individually between myself and Christine significant morphological variation was observed. Statistical analysis ANOVA and t-test carried out by myself compared treated and control plants. Plants were compared only within the line and not between species because of their morphologically diversity. Different lines were grouped and consider as being resilience (tolerance) and or susceptible. After the data was sorted and ranked according to the level of significance (p-values). Based on the ranking, an improvement in leaf area against the control was chosen as a marker to guide selection. This work led to the selection of 13 lines of susceptible and tolerant plants (26 in total) were selected from the 52 (**Table 1.2**). These were the lines selected to be taken forward within my main thesis. However, some of the wild S1 lines had issue with seeds availability and some on poor germination (see Table 2.1 in Chapter 2 for the list of the retained ones). These were subsequently dropped.

Table 1.2: List of some selected *B. oleracea* genotype lines from 2014 salt stress experiment

Tolerant			Susceptible		
line no.	Geno ID	Name	line no.	Geno ID	Name
2	C04099	<i>B. oleracea</i> (DHSL150 C01001)	5	C07011	<i>B. cretica</i>
3	C07007	<i>B. bourgaei</i>	8	C07015	<i>B. cretica</i>
4	C07010	<i>B. cretica</i>	11	C07019	<i>B. hilarionis</i>
25	C07059	<i>B. oleracea</i>	12	C07020	<i>B. hilarionis</i>
26	C07060	<i>B. oleracea</i>	14	C07024	<i>B. incana</i>
32	C07073	<i>B. oleracea</i>	20	C07045	<i>B. macrocarpa</i>
33	C07075	<i>B. oleracea</i>	22	C07047	<i>B. macrocarpa</i>
35	C07078	<i>B. oleracea</i>	23	C07049	<i>B. macrocarpa</i>
36	C07079	<i>B. oleracea</i>	29	C07069	<i>B. oleracea</i>
41	C07091	<i>B. incana</i> (listed as villosa in seed book)	37	C07079A	<i>B. oleracea</i>
43	C07094	<i>B. incana</i>	46	C07104	<i>B. villosa bivoniana</i>
47	C07113	<i>B. villosa tinei</i>	49	Early Big	<i>B. oleracea</i>
51	HRIGRU008	<i>B. oleracea</i>	48	C07123	<i>B. oleracea capitata</i>

***Note:** The listed *B. oleracea* in brown colour Geno ID are those that show an ability to grow (Tolerant) to short-term salt stress based on; Plant Height, Plant dry weight, leaf dry weight and leaf area as compared to the control While those in green colour are considered susceptible to short-term salt stress.

1.6.2 Salt Stress Screening Experiments in 2015

During the initial experiment work by Peter Walley and G. Teakle, generated DH lines derived from the original wild founder DFS (section 1.3.5.1) lines and related S1 lines (Table 2.3 in chapter 2). This offered a valuable resource and lines related to the wild lines selected were identified and seeds obtained. Another valuable addition enabling me to develop my work arose from transcriptomic profiling using RNA-Seq analysis conducted on three (3) of the DH lines and one parent line (DHSL150). Two DH lines, C13013 & C10128 were selected for resilience while C13001 & DHSL150 were considered as susceptible. Christine Hicks reported the data in her final thesis this is the reason I have not included this work in my main thesis although she used plant materials (DH lines and DHSL150), from my initial salt stress experiments. Based on this fact, it was recommended that I should review that aspect of her work and come up with my own hypothesis. After this review, I concentrated on the iron membrane proteins that were shown to be expressed in some DH lines and significantly different from the tolerant and susceptible lines. The hypothesis was that expression of these membrane ion transporters would increase in *B.*

oleracea genotypes tolerant lines enhances resilience as compared to susceptible genotypes. The 2017 the data (Fastq files) were re-analysed and the Fastq files were aligned to *B. oleracea* (To1000) genome. I kindly appreciate Luca Illing for his support in guiding me through this. Following the alignment, heat-reads (BAM files) were further processed using SeqMonk software, by using two statistical algorithms; DSeq2 and Edge R analyses. This formed the basis of the list of iron membrane transporters that were subsequently investigated (Chapter 5).

1.7 AIM AND OBJECTIVES

1.7.1 The aim of the Work is:

To study variation in salt stress response in some selected *B. oleracea* genotypes including the founder rapid cycling DHSL150, the wild DFFS S1 and their derived doubled haploid (DH) lines and try to identify the allele(s) that confer beneficial trait(s) in response to salt stress.

1.7.2 Objectives are:

- (i) To establish the extent of phenotypic variation in response to salt shock stress in *Brassica oleracea* genetic collections;
- (ii) To determine the difference in the mineral content of diverse *Brassica oleracea* accessions in response to a salt shock to identify the range of pathways potentially involved.
- (iii) To verify the effect of genotype and environmental factors in regulating the plant's response to salt shock;
- (iv) To employ bioinformatics tools to study the variation in salt shock responsive genes and to determine if the same pathways are shared in salt tolerant lines.
- (v) To utilize the functional genomics studies to understand the relationship between the DHs derived from the S1 material, deduce the length of the introgression and consequently map out genes which might underlie salt shock tolerance in *Brassica oleracea*.

CHAPTER TWO

MATERIAL AND METHODS

2.0 Material

2.0.1 Description of genetic relationship between the plants used for salt shock experiment

The *B. oleracea* genotypes used in this work are the cultivated rapid cycling founder line *B. oleracea* (DHSL150) and wildtype S1 plants, they are members of specific core collection of *B. oleracea* C-genome named Diversity Fixed Foundation Sets (DFSS) "representing an informative set of genetically fixed lines, and a structured sampling of diversity across a gene pool" already described in Chapter 1, under the Warwick Crop Centre, Warwick Horticulture Research International (HRI), Warwick University UK.

The doubled haploid (DH) lines were derived by microspore tissue culture of F1s-hybrids generated from crosses between different accession of wildtype and cultivated rapid cycle line DHSL150, These lines were selected as already discussed and listed in (**Table 2.1a**) were used for 2015 salt screening experiment and **Table 2.1b** for 2017. Different F1s were generated and from the haploid F1s-hybrid material, the DH lines were derived by using the microspore tissue culture and chromosomes diploidised by an anti-microtubule agent colchicine (Chapter 1 & **Figure 1.3**). The DH lines produced were allowed to selfed where seeds from individual related line were collected together.

Table 2.1a: List of the selected *B. oleracea* S1s and their related DH lines used in 2015 salt stress screening experiment

Line	Line ID	Line Name	Line Type	Not Used in the screening Founder wild DFS (P2)
A	C10001	DHSL150	DH (P1)	
B	C07007	<i>B. bourga</i>	S1	C04006
C	C10025		DH	C04006
D	C10027		DH	C04006
E	C07060	<i>B. olerace</i>	S1	C04052
F	C10125		DH	C04052
G	C10128		DH	C04052
H	C07094	<i>B. incana</i>	S1	C04081
I	C13012		DH	C04081
J	C13013		DH	C04081
K	C07019	<i>B. hilaris</i>	S1	C04015
L	C13001		DH	C04015
M	C07069	<i>B. olerac</i>	S1	C04062
N	C10132		DH	C04062
O	C10139		DH	C04062
P	C07079A	<i>B. olerac</i>	S1	C04069
Q	C10121		DH	C04069

* **Note:** DH lines are put beneath to any S1 line as their related parent. DFS wild founder lines never being used in this experiment.

Table 2.1b: List of *B. oleracea* lines used in 2017 for salt stress experiment

Line	Line ID	Line Name	Line Type
A	C10001	DHSL150	DH
B	C07007	<i>B. bourga</i>	S1
C	C10025		DH
E	C07060	<i>B. olerace</i>	S1
G	C10128		DH
J	C13013		DH
L	C13001		DH
P	C07079A	<i>B. olerac</i>	S1
Q	C10121		DH

* **Note:** Some wild S1 lines and DH lines were dropped in 2017 because of no enough seeds or due to poor germination.

2.0.2 Seeds Collection and Seeds Plantation

Seeds were collected from the VeGIN genetic resources, Warwick Crop Centre, Wellesbourne Campus, The University of Warwick, the UK sourced on the 11th May 2017. This remains the only source of the seeds being used throughout the salt stress experiment.

2.0.3 Composition of M2 Compost Soil Used

M2 compost has been designed to suit the growth of a wide variety of bedding plants. It provides a good quality nutrient supply, and the physicochemical constituents includes; pH – 5.3 – 6.0, nitrogen (N) – 192 mg/L, phosphorus (P) – 98 mg/L, and potassium (K) – 319 mg/L and finally, with particle – size of between 0 – 10 mm respectively.

2.0.4 Glasshouse Conditions

The glasshouse was made to ensure that the average temperature was maintained at 20⁰ C day and 18⁰ C night (**Figure 2.1**). The plants were supplemented with available light (400 W SONT lamps), photoperiod was set at 03:00 – 19:00 hrs to ensure photosynthesis. The plants were also maintained under reliable controlled internal environment, containing air handling and drainage for the enhancement of natural light penetration, and reduced pest control.



Figure 2.1: Phytobiology Facility, Glasshouse, The University of Warwick, UK

2.0.5 Experimental Design

A Completely Randomised Design (CRD) was applied in three replications (**Figures 2.2 & 2.3**), control and treated plants were paired. The plants were adequately watered throughout the experiment.

	C07094-Salt	C07094-Ctrl	C13013-Ctrl	C13013-Salt	C07007-Ctrl	C07007-Salt	C07060-Ctrl	C07060-Salt	C10025S-Salt	C10025-Ctrl
	C07019-Salt	C07019-Ctrl	C07079A-Ctrl	C07079A-Salt	DHLS150-Ctrl	DHLS150-Salt	DHLS150-Ctrl	DHLS150-Salt	C07094-Salt	C07094-Ctrl
	C07060-Salt	C07060-Ctrl	C10121-Ctrl	C10121-Salt	C07019-Ctrl	C07019-Salt	C07019-Ctrl	C07019-Salt	C10128-Salt	C10128-Ctrl
REP 1	C13013-Salt	C13013-Ctrl	C10025-Ctrl	C10025-Salt	C07079A-Ctrl	C07079A-Salt	C13001-Ctrl	C13001-Salt	C10121-Salt	C10121-Ctrl
	C10025-Salt	C10025-Ctrl	C10128-Ctrl	C10128-Salt	C07060-Ctrl	C07060-Salt	C10121-Ctrl	C10121-Salt	C13001-Salt	C13001-Ctrl
	C10128-Salt	C10128-Ctrl	C07007-Ctrl	C07007-Salt	C10121-Ctrl	C10121-Salt	C07094-Ctrl	C07094-Salt	C07019-Salt	C07019-Ctrl
	C13001-Salt	C13001-Ctrl	DHLS150-Ctrl	DHLS150-Salt	C07094-Ctrl	C07094-Salt	C07007-Ctrl	C07007-Salt	C07007-Salt	C07007-Ctrl
	C07079A-Salt	C07079A-Ctrl	C07094-Ctrl	C07094-Salt	C10128-Ctrl	C10128-Salt	C10128-Ctrl	C10128-Salt	C07079A-Salt	C07079A-Ctrl
	C07007-Salt	C07007-Ctrl	C07019-Ctrl	C07019-Salt	C13013-Ctrl	C13013-Salt	C10025-Ctrl	C10025-Salt	DHLS150-Salt	DHLS150-Ctrl
	DHLS150-Salt	DHLS150-Ctrl	C13001-Ctrl	C13001-Salt	C10025-Ctrl	C10025-Salt	C07079A-Ctrl	C07079A-Salt	C13013-Salt	C13013-Ctrl
	C10121-Salt	C10121-Ctrl	C07060-Ctrl	C07060-Salt	C13001-Ctrl	C13001-Salt	C13013-Ctrl	C13013-Salt	C07060-Salt	C07060-Ctrl
	C13001-Ctrl	C13001-Salt	C07007-Salt	C07007-Ctrl	C10025-Ctrl	C10025-Salt	C10128-Salt	C10128-Ctrl	C07019-Ctrl	C07019-Salt
	C07060-Ctrl	C07060-Salt	C07079A-Ctrl	C07079A-Salt	C07094-Ctrl	C07094-Salt	DHLS150-Salt	DHLS150-Ctrl	C07007-Ctrl	C07007-Salt
	C07079A-Ctrl	C07079A-Salt	C07094-Ctrl	C07094-Salt	C10128-Ctrl	C10128-Salt	C13013-Salt	C13013-Ctrl	C13013-Ctrl	C13013-Salt
	C10121-Ctrl	C10121-Salt	C13001-Salt	C13001-Ctrl	C07079A-Ctrl	C07079A-Salt	C07007-Salt	C07007-Ctrl	C10121-Ctrl	C10121-Salt
REP 2	C13013-Ctrl	C13013-Salt	C07060-Salt	C07060-Ctrl	C13013-Ctrl	C13013-Salt	C07079A-Salt	C07079A-Ctrl	DHLS150-Ctrl	DHLS150-Salt
	C07094-Ctrl	C07094-Salt	DHLS150-Salt	DHLS150-Ctrl	C10121-Ctrl	C10121-Salt	C07019-Salt	C07019-Ctrl	C07094-Ctrl	C07094-Salt
	C10025-Ctrl	C10025-Salt	C10025-Salt	C10025-Ctrl	C07019-Ctrl	C07019-Salt	C10025-Salt	C10025-Ctrl	C10128-Ctrl	C10128-Salt
	C10128-Ctrl	C10128-Salt	C07019-Salt	C07019-Ctrl	C07060-Ctrl	C07060-Salt	C07060-Salt	C07060-Ctrl	C10025-Ctrl	C10025-Salt
	C07019-Ctrl	C07019-Salt	C10128-Salt	C10128-Ctrl	C07007-Ctrl	C07007-Salt	C10121-Salt	C10121-Ctrl	C07079A-Ctrl	C07079A-Salt
	C07007-Ctrl	C07007-Salt	C10121-Salt	C10121-Ctrl	DHLS150-Ctrl	DHLS150-Salt	C13001-Salt	C13001-Ctrl	C13001-Ctrl	C13001-Salt
	C07019-Salt	C07019-Ctrl	C07060-Ctrl	C07060-Salt	C13013-Salt	C13013-Ctrl	C07019-Ctrl	C07019-Salt	C07060-Salt	C07060-Ctrl
	C13013-Salt	C13013-Ctrl	C07007-Salt	C07007-Ctrl	DHLS150-Salt	DHLS150-Ctrl	C13001-Ctrl	C13001-Salt	DHLS150-Salt	DHLS150-Ctrl
	C10128-Salt	C10128-Ctrl	C10121-Ctrl	C10121-Salt	C07079A-Salt	C07079A-Ctrl	C10128-Ctrl	C10128-Salt	C07007-Salt	C07007-Ctrl
	C10121-Salt	C10121-Ctrl	C13013-Ctrl	C13013-Salt	C07060-Salt	C07060-Ctrl	C13013-Ctrl	C13013-Salt	C07094-Salt	C07094-Ctrl
REP 3	C07060-Salt	C07060-Ctrl	C10128-Ctrl	C10128-Salt	C10121-Salt	C10121-Ctrl	C07060-Ctrl	C07060-Salt	C10128-Salt	C10128-Ctrl
	C10025-Salt	C10025-Ctrl	C10025-Ctrl	C10025-Salt	C07007-Salt	C07007-Ctrl	C10025-Ctrl	C10025-Salt	C07079A-Salt	C07079A-Ctrl
	C07079A-Salt	C07079A-Ctrl	C07094-Ctrl	C07094-Salt	C07019-Salt	C07019-Ctrl	DHLS150-Ctrl	DHLS150-Salt	C10025-Salt	C10025-Ctrl
	DHLS150-Salt	DHLS150-Ctrl	C07019-Ctrl	C07019-Salt	C13001-Salt	C13001-Ctrl	C07079A-Ctrl	C07079A-Salt	C13013-Salt	C13013-Ctrl
	C07094-Salt	C07094-Ctrl	DHLS150-Ctrl	DHLS150-Salt	C07094-Salt	C07094-Ctrl	C07007-Ctrl	C07007-Salt	C10121-Salt	C10121-Ctrl
	C07007-Salt	C07007-Ctrl	C07079A-Ctrl	C07079A-Salt	C10025-Salt	C10025-Ctrl	C07094-Ctrl	C07094-Salt	C13001-Salt	C13001-Ctrl
	C13001-Salt	C13001-Ctrl	C13001-Ctrl	C13001-Salt	C10128-Salt	C10128-Ctrl	C10121-Ctrl	C10121-Salt	C07019-Salt	C07019-Ctrl

Figure 2.2: A completely randomised design salt stress experiment. Different colours used to represent individual lines. Control and treated are paired and distributed in 4 x 4 square meter (m²) space.



Figure 2.3: A completely randomised design and well-spaced salt shock experiment used to Induced Salt tress on *B. oleracea* plants.

2.0.6 Seed germination

The seed germination was monitored 6 – 7 days after seeds were sown and subsequently on a daily basis for three weeks (**Figure 2.4**). Lines with early germination were noted; likewise, those with late germination for proper placement into the experimental design as according to the age of the plant and number of leaves counted. After four weeks, healthy plants were selected and transplanted into bigger pots. The poorly growing plants were allowed two additional weeks before being selected.

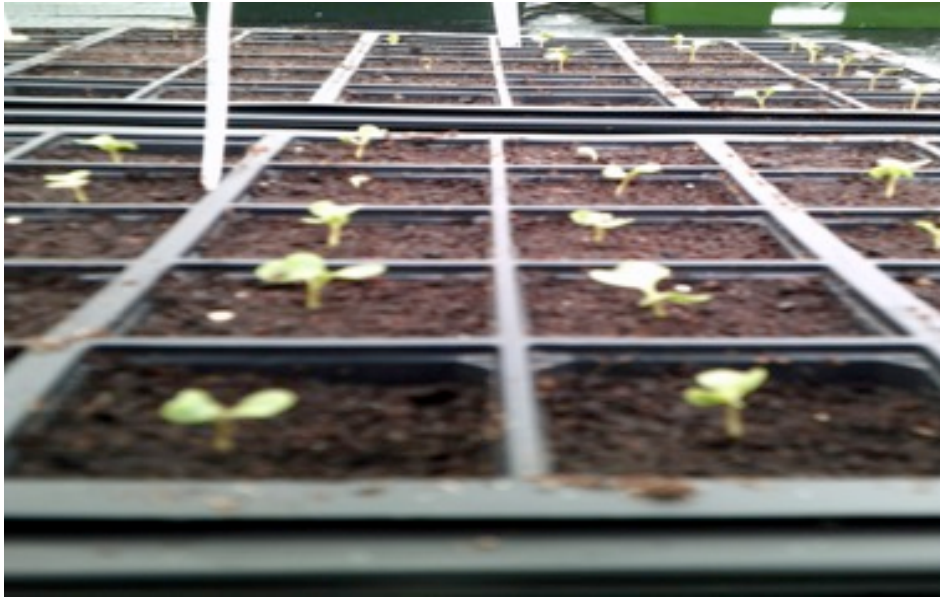


Figure 2.4: A set of 7 days germinated DHSL150 *Brassica oleracea* lines in a 4 X 10 tray setting one-week after sowing.

2.0.7 Plant growth

Plants growth was monitored for two additional weeks following transplantation for standard and rapid growth before a final selection at six weeks (4th – 5th leaf appeared) (**Figure 2.5 & 2.6**) those at an appropriate stage of growth and the lines with sufficient germination were divided into three reps each both for control and treated for the experiment. Unselected plants were transferred to Wellesbourne Campus as spare for seed generation.



Figure 2.5: Young growing sets of DHSL150 *Brassica oleracea* genotypes. A) 2 week old B) 4 weeks old and C) transplanted DHSL150 into bigger pots.

2.1 Methods

2.1.2 Salt Shock Induction

This was carried out when the plants were at week six (6) of growth (i.e., at least each plant had five full expanded leaves). This was done using a freshly prepared 250 mM NaCl, and treated plants were fed with an equivalent of 200 mL using a 500 mL measuring cylinder. The control plants were supplied with an equivalent of 200 mL of running tap water. **Note:** A plastic span was put under each pot to prevent water leakages and possible salt leaking, and this was allowed till the end of the experiment.



Figure 2.6: The growing *B. oleracea* selected wild S1, DHSL150 and doubled haploid lines set up for salt induction at six weeks old.

2.1.2 Sample Collection and Storage of Plant Material

Samples were collected twenty-four hours (24 hr) after the salt shock induction for both RNA extraction and mineral analysis. For RNA extraction, a disc-shaped portion of leaf four (4) was taken from both treated and control in an Eppendorf tube, flash frozen in liquid nitrogen and stored at -80 °C. For mineral analysis, a more substantial portion of the same leaf (#4) (~ 0.5g) was stored in well-labelled paper bags and oven dried overnight at 80° C for 12 hours at Crop Centre, Wellesbourne Campus.

Note: Sample collection was also repeated at these other stages of the experiment:

- 1) Two-weeks post-salt shock induction (8 weeks old); (leaf 5 collected)
- 2) A second salt shock was carried out a day after (1) above and induction procedure was as same as the first induction and sample collected 24 hours (leaf 6 collected) and finally;
- 3) Two-weeks post second induction (10 weeks old) (leaf 7 collected)

All samples were kept under the same condition until analyses.

2.1.3 Plant height

Plant height was measured at weekly intervals after the plants were salty shocked. The measurement was done using a flexible measuring tape, so as to make sure bent plants were measured correctly from an upper edge of the pot serves as the baseline to maintain an equal starting point, and measurement was done from the baseline to the tip of the stem for each experimental plant in centimetre (cm).

2.1.4 Plant fresh/dry weight

Plant fresh weight was determined at collection, i.e., at tenth weeks. The whole plant (flowers, stems, and leaves) were collected, harvested using scissors from a position above the compost soil and carefully folded and placed in a pre-labelled paper bags for each plant

(i.e., one each of the three control and same for three treated) and stored in the cold room temporarily. The harvested plants in paper bags were subsequently moved to Crop Centre at Wellesbourne and oven dried at 80⁰ C for 12 hrs. The dry weight was measured using a digital weighing balance set at grams (g) position, an empty paper bag used to zero the machine and finally weighing the plants' materials.

2.1.5 Leaf fresh/dry weight

To measure the fresh weight, true leaf seven (**leaf #7**) was collected at the tenth week of the experiment. The leaves were taken by incision from the petiole, at a point of attachment to the stem. The individual leaves were packed in a paper bag, pre-labelled, and measured, kept in cold room before being moved to Wellesbourne where they were oven dried at 80⁰ C for 12 hrs and dry weight measured in grams (g).

2.1.6 Leaf Area

Leaf seven (#7) collected was carefully photographed using a Canon camera in the darkroom, the camera adjusted to about 50 cm above the base where the leaf was put down on a white A4 paper as a background and a meter rule used for scale included. The zoom of the camera and focus were carefully maintained, and paired control and treated leaves were photographed for the three reps. The pictures were used to calculates the leaf area using a publicly available software **Image J** set at cm² unit.



Figure 2.7: Morphological differences between the parent and their derived DH *B. oleracea* genotypes. A) *B. bourgaei-S1* (B) DHSL150, (C) DH (C10025-DH), and D) DH (C10027-DH).

2.1.7 Mineral Analysis

2.1.7.1 Material

Plant tissue used (dried leaves) (see Section 2.2.2)

2.1.7.2 Chemicals

Nitric acid 67% HNO₃ (w/w): *Plasma* PURE, 250-039-171 (SCP SCIENCE). Multi-element standard (Ca 99.1 ± 0.8 µg/ml, K 100.3 ± 0.9 µg/ml, and Na 101.2 ± 1.0 µg/ml) and Matrix of 5.0% HNO₃ (ICP – AES), Cat Number: AQ0-053-621, (SCP SCIENCE)

2.1.7.3 Sample Handling and Preparations

Before analysis, samples were first dried overnight (12 hr) at 80°C, and processed according to the modified procedure of Campbell and Plank (1992). A dried tissue sample (~ 0.5g) was ground into smaller pieces to increase digestion efficiency. The dried tissue samples were placed into standard 50 mL PTFE digestion tubes to which 2 mL of 67% HNO₃ (w/w) added and mixed for about 10 sec before loading the containers into microwave digestion system (MARS 5 CEM Corporation, USA). The microwave *MARSX* (**Figure 2.8**) digestion machine was set up using programme heating cycles for 34 minutes as presented in **Table 2.2**.

Table 2.2: A microwave digestion system set-up programmed heating cycles.

Cycle	Initial Temp ($^{\circ}\text{C}$)	Time Interval (min)	Final Temp ($^{\circ}\text{C}$)	Hold time (min)
1	0	3	100	2
2	100	1	120	1
3	120	3	160	2
4	160	2	180	20

After the digestion, the tubes were allowed for 30 min of cooling time, and 48 mL of deionised water added to each vessel. (Note: sample filtration was performed on the partially digested sample to remove any undigested plant material).



Figure 2.8: *MARSX* microwave digestion system, Warwick Crop Centre, Wellesbourne, UK.

2.1.7.4 Sample dilution

Following successful digestion, samples were further diluted 1:20 using a 20-fold dilution factor (i.e., 1 mL in 19 mL of deionized water), this was to bring closer the matrix of the sample and that of the standard while running in ICP – MS, as recommended by the Chemistry Dept. The University of Warwick, where the samples were run.

2.1.7.5 Preparation of Standard Curve Solution

A standard solution was prepared from the multi-element standard (Ref: Number: AQ0-053-621, SCP Science) in order to establish a standard curve from which the unknown sample concentrations could be extrapolated. Serial dilution of the stock to make the standard curve included a concentration range from 0.00, 0.039, 0.078, 0.156 10 ppm were prepared and run under the programme listed in Table 2.3. **Note:** standard curve (see Appendix III) was prepared freshly and established on daily basis to ascertain

reproducibility before running the samples. The samples were analysed using an Agilent - ICP – MS in the Department of chemistry, University of Warwick, the UK in 2017.

2.1.7.6 Sample Running

After the establishment of the standard curve, samples were run by using the machine conditions outlined in **Table 2.3** below and **Figure 2.9**.

Table 2.3: ICP-MS Agilent 7500 series programme for sample injection during mineral analysis

Instrument/Method Details		Instrument Usage		
Instrument:	Agilent 7500cx ICP-MS	Plasma On	Plasma Off	Instrument time used
Plasma Gas:	Argon	0.416667	0.708333	0.291667
Plasma Gas Flow Rate:	15L/min	0.475694	0.625	0.149306
Forward Power:	1550W	0.402778	0.638889	0.236111
Spray Chamber Temp:	15degC	0.402778	0.597222	0.194444
Internal Standard:	^{166}Er			
			Total	0.871528
Total Analysis				
Standards	40			
Samples	228			
Total	268			

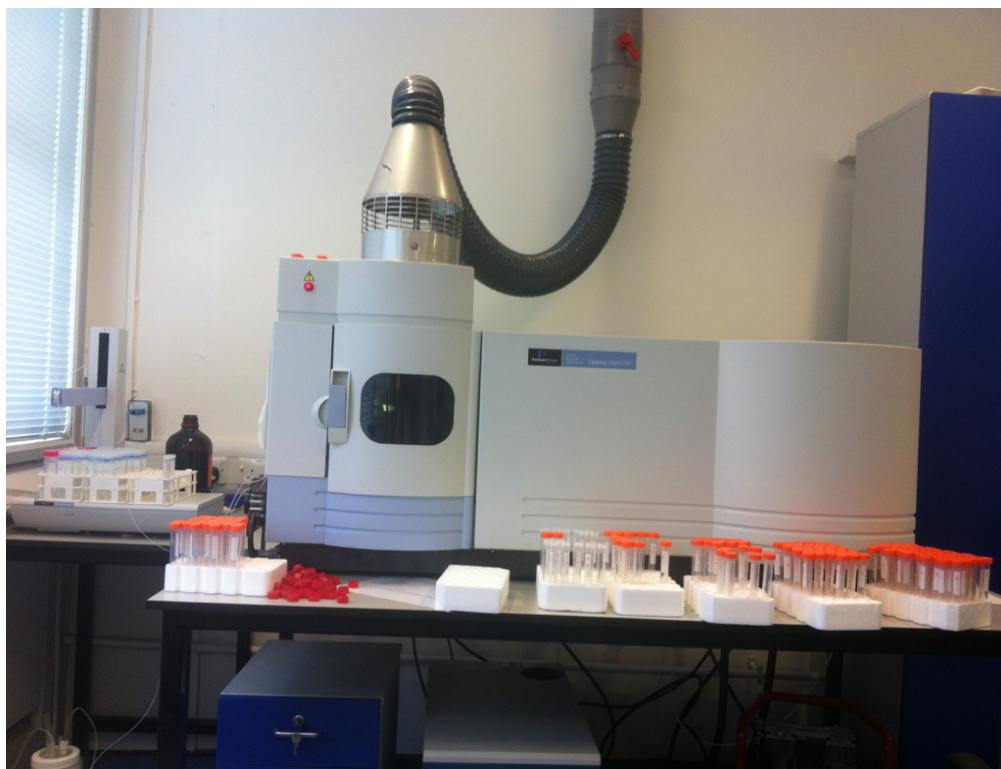


Figure 2.9: Already set up Agilent Technologies ICP-MS 7500 series for determination of leaf mineral content in *B. oleracea* leaves. Department of Chemistry, University of Warwick, UK.

2.1.8 qPCR

2.1.8.1 RNA Extraction and Purification

RNA extraction was carried out using an RNeasy Plant mini kit (Qiagen). Firstly, leaf tissue (see Section 2.2.2) of disc shape (1cm size) was thoroughly ground into a fine powder in liquid nitrogen using a cleaned, sterilised and cooled mortar and pestle. The grounded leaf material was carefully decanted into 2 mL Eppendorf, and 450 µl (RLT buffer) was added, which was vortexed vigorously. The lysate was transferred into a (QIAshredder) spin column and placed into newly 2 mL collection tube. The tube was centrifuged at 14000 x g for 2 min (Eppendorf microcentrifuge) and the supernatant was carefully transferred into a newly Eppendorf (care was taken not to disturb the lysate). A 0.5 volume of ethanol (96 – 100%) was added to the cleared lysate and gently mixed. The mixture was then transferred onto an RNeasy mini spin column and centrifuged at 8000 x g for 15 sec and the flow-through was discarded. A 700 µl (RW1 buffer) was then added into the RNeasy spin column, centrifugation was repeated and the flow-through discarded. A 500 µl (RPE buffer) was then added onto the RNeasy spin column and centrifuged at 8000 x g for 2 min. The RNeasy spin column was then transferred into a 2 ml collection tube, which was centrifuged at maximum speed to remove any remaining buffer from the column membrane. And finally, the RNA was eluted by using 25 µl of RNase-free water.

2.1.8.2 cDNA Synthesis Protocol

Prior to the cDNA synthesis, water bath and block air incubator were pre-heated and maintained at 65⁰ C and 42⁰ C respectively. An equivalent volume representing 2.5 µg of the extracted mRNA was used for cDNA synthesis.

Two masters mixed were prepared; the first one was made in a 1.5 ml Eppendorf tube which contained an equivalent of 2.5 µg total mRNA, 1µl oligo (dT) primer (20 mM) and molecular water used to make up the volume to 20 µl. The content was mixed and centrifuged briefly and incubated at 65⁰ C for 5 min. The second master-mixed was prepared using 10.0 µl 5xRT buffer, 5.0 µl DTT (20 mM), 1.0 µl of premixed dNTPs and 0.5 µl RT Superscript II respectively. The tubes were placed on ice for 2 min before being placed in the water bath at 65⁰ C. A 16.5 µl of the second prepared master-mixed was pipetted into each tube, and the final volume brought up 50 µl with molecular water and mixed. The tubes were then incubated at 42⁰ C for 1 hr after which the cDNA synthesised was diluted with 200 µl of molecular water and kept at -20⁰ C until required.

2.1.8.3 List of Genes, Primer design and Testing

These are selected ion membrane transporter transcripts that showed significant variation in differential expression in both tolerant and susceptible lines obtained from RNA-Seq data were chosen for qPCR validation (**Table 2.5**).

Transcript-specific primers were designed to amplify a specific cDNA sequence of the transcripts in our samples. The sequences of mRNAs of the transcripts of interest by using their individual transcripts IDs were downloaded from (https://plants.ensembl.org/Brassica_oleracea/) and further

(<https://www.ncbi.nlm.nih.gov/blast/>) to use for primer design. The primers were designed using DNASTAR (Lasergene 14) software. The parameters were used as follows: T_m , 55°C to 62°C, differences not >2°C between the primers in a pair was insured; primer length, 19-24 bp; GC content, 45-55%; amplicon length, 100-150 bp. Whenever possible, primers were designed to span introns and insured that only primers yielding a single product in conventional PCR and qPCR were used in the validation (**Table 2.4**).

Table 2.4: List of genes and their mRNA primer sequence used for qPCR validation Analysis

Gene stable ID	Gene name	Gene description	Forward Sequence	Reverse Sequence
Bo1g022080	KT	Potassium transporter	TGGCGGAAAGGGTAGAAACAT	TGGATGAAGAAGCTACTAAG
Bo1g158860	V-type G	V-type proton ATPase subunit G	CCAGCAGAGGAGGAGGAGGTG	TTCGATTTTGGCATCAGTCTCTTG
Bo2g024320	V-type a	V-type proton ATPase subunit a	AATGGCTCCACTGCAACTTCTC	GTTTTCATTTTCACTTTATCGCT
Bo4g012670	KT 9	Potassium transporter 9	TGGTGTCTGTCTTTTCGTTTCTG	TGATAACGAGAAGGTGGGACT
Bo4g039050	KUP11	K ⁺ uptake permease 11	ACAATGGGTGGTGGTGGTGA	TATGGATGAAGAAGCTGGTCGG
Bo4g145930	V-type a	V-type proton ATPase subunit a	GGTGGTGGTGGTGGTGGT	TGCTCATCTCACCGCTCTTACCT
Bo5g131740	CAX3	cation exchanger 3	AGATGTCCAAACCCGCCGTCAG	GATTCTCCTCGGCCGACGGTT
Bo8g030800	V-CLC	Voltage-gated chloride channel family protein	TCTTGCTACGAGCTCTCCAGTCCT	AACGGCGAGGTTGTTGGTAAA
Bo9g003910	ECA2	ER-type Ca ²⁺ -ATPase 2	GAAGTCTTTATCTCCGTGGTCGTG	GATGTCCAAACCCGCCGTCAG
Bo9g010200	NHX1	Na ⁺ /H ⁺ antiporter	TCGTTTTGGATTCTTTTCGTAT	GTATTGTCATTGCCCATCTCTTGG
Li61-qRNA-Btub	B-tubulin	Housekeeping Gene	TCATGGATCTGGAGCCTGGAAC	GGAATGGCAAACCTGAAACCC
TIP41	TIP41	Housekeeping Gene	CACCGGAAGCCTCTGACTGAT	TGATGGTGTGCTTATGAGGTGAG

2.1.8.4 Primer Testing, PCR and Gel running

Conventional PCR using touch-down procedure was carried out to test the primers to ensure that only the required sequence was produced for a pair of primers and the required amplicon length. The PCR was carried out using the setup procedure; a master mix of 12.5 µl RedTag, 5.5 µl DH₂O and 5.0 µl cDNA (23 µl total) was prepared for individual genes and 1 µl each for a pair of forward and reverse primers were added to make the total amplicon 25 µl. PCR thermocycling was set using the parameters; denatured temperature, 95°C for 5 min; annealing temperatures, 62°C - 55°C for 1.0 min and 72°C for 30 sec and x 30 cycles; and hold at 4°C for 5 min. The product was run 1% agarose gel as shown in **Figure 2.10**.

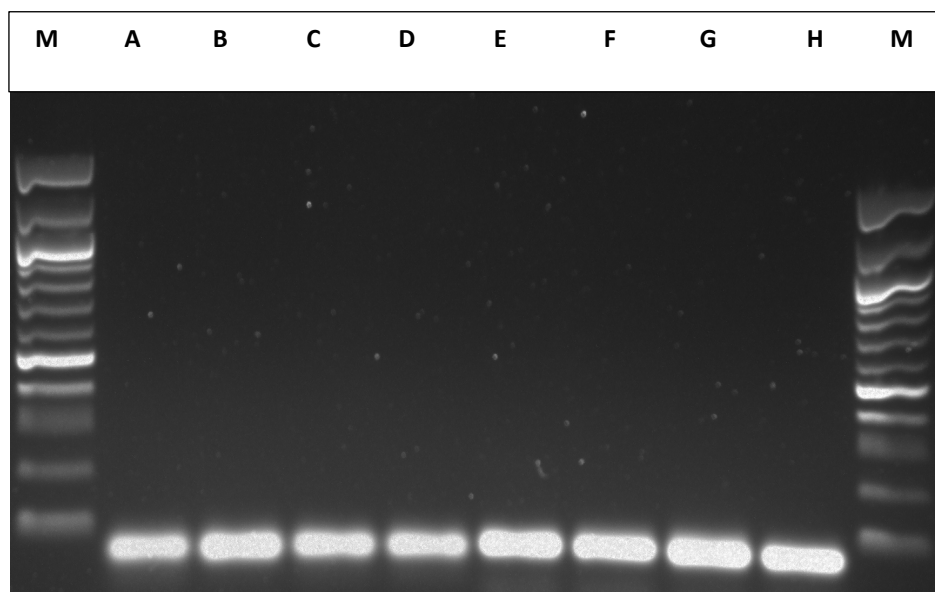


Figure 2.10: Analysis of quality assay results. A 5.0 μ l of each PCR products were electrophoresed on a 1% agarose gel/RedTag gel in 2XTAE buffer following 40 cycles of qPCR. Lane **M**: 100 bp DNA Ladder size marker, **A – H** indicate the bands at 100 bp, representing the 3' – 5' fragments of a primer pair of one of the candidate gene. **A – D** = non-treated control and **E – H** = treated samples.

2.1.8.5 Sanger sequencing

The PCR amplified product was further cleaned up using a QIAGEN quick PCR purification kit and the procedure followed was based on the manufacturer's guidelines. After the clean-up, 5 μ l of μ M each of forward and reverse primers were premixed in separate 1.5 Eppendorf's tubes containing 5 μ l of template were sent for GATC sequencing (<https://warwick.ac.uk/fac/sci/lifesci/intranet/staffpg/support/...sequencing.../gatc/>). **Note:** only forward and reverse primers that produced correct clean sequences were used for qPCR validation.

2.1.8.6 Plate preparation and qPCR running

5.0 µl cDNA samples prepared from the total RNA extracted from three biological replicates of the experimental plants were required. A master mix was prepared using 10.0 µl SYBGREEN as a detection probe and 2.5 µl each of primer pair (Forward and Reverse). Plate set-up was prepared using a randomised design (**Figure 2.11**), each colour code represents control and treated samples replicated in randomly, each sample was included three-times per primer pair or with the two housekeeping primer genes (β -Tubulin and TIP41).

	1	2	3	4	5	6	7	8
A	A1CLC	C3CLC	E5CLC	A7Bt	C9Bt	E11Bt	E17IP41	H20ITP41
B	A1CLC	C3CLC	E5CLC	A7Bt	C9Bt	E11Bt	E17ITP41	
C	A1CLC	C3CLC	E5CLC	A7Bt	C9Bt	E11Bt	E17IP41	
D	B2CLC	D4CLC	F6CLC	B8Bt	D10Bt	F12Bt	F18ITP41	
E	B2CLC	D4CLC	F6CLC	B8Bt	D10Bt	F12Bt	F18ITP41	
F	B2CLC	D4CLC	F6CLC	B8Bt	D10Bt	F12Bt	F18ITP41	
G	A13ITP41	A13ITP41	A13ITP41	C15ITP41	C15ITP41	C15ITP41	H20CLC	
H	B14ITP41	B14ITP41	B14ITP41	D16ITP41	D16ITP41	D16ITP41	H20Bt	

Figure 2.11: A randomise qPCR design. Colour code indicates a particular primer pair position.

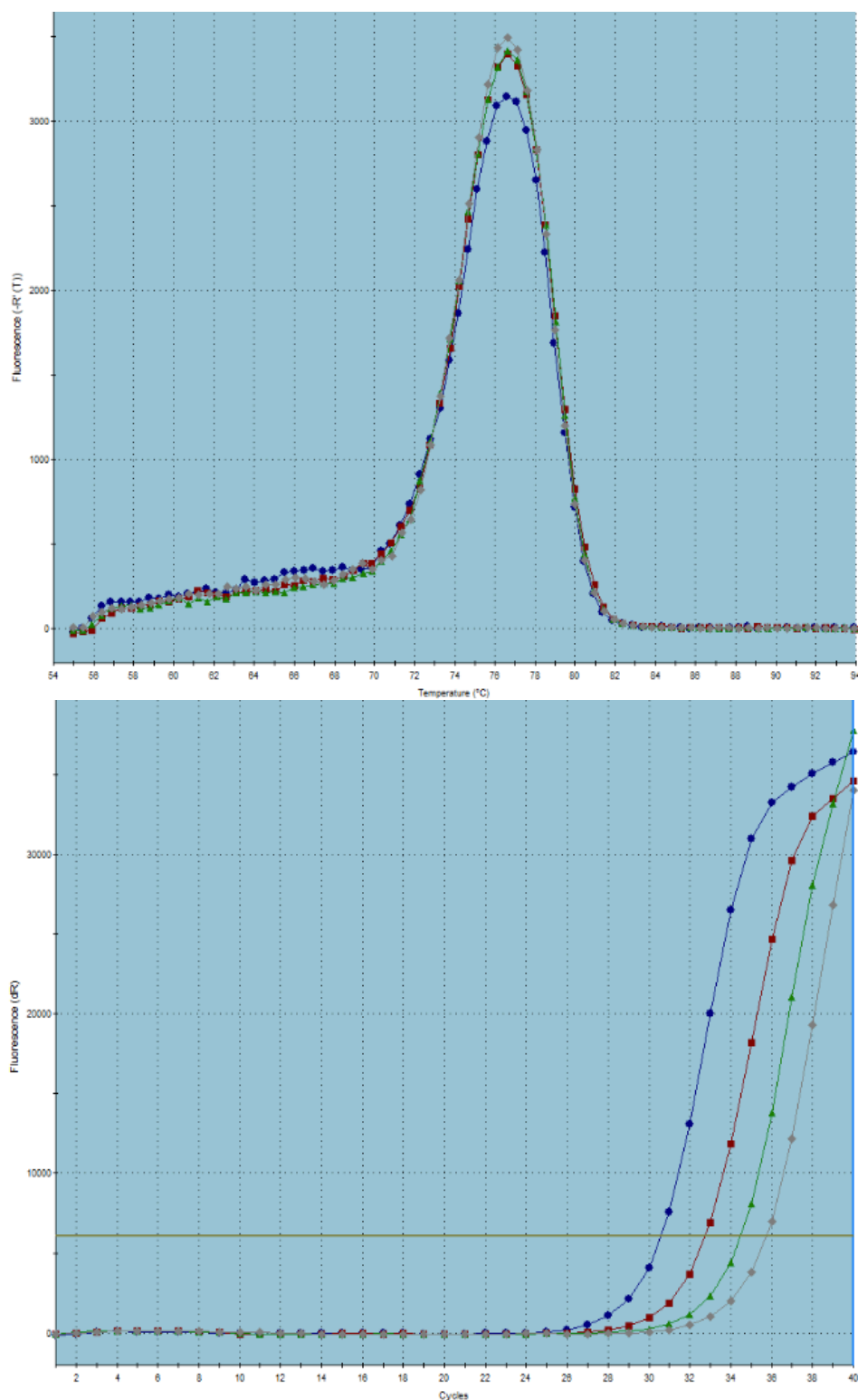


Figure 2.12: A schematic representation (a) a dissociation curve by using *V-CLC* primer pair listed on **Table 2.4** at different cDNA concentration to ensure the working cDNA is free from contamination and the primer pair amplify only product of interest (b) an amplification curve at different cDNA concentration: cDNA was prepared in 10-fold serial dilutions to establish the amplification curve to further confirm the workability of the probe used (SYBGREEN).

2.1.9 Genotyping by sequencing

2.1.9.1 Sample Collection

A disc of leaf sample of approximate size of 1cm² was collected from ninety-six (96) *B. oleracea* accession from the C genome diversity set from true leaf seven when the plants were approx. 2 months old. (<http://www2.warwick.ac.uk/fac/sci/lifesci/research/vegin/brassica/bcgdfbs/bcgdfbslinessu mm.pdf>) by Hussien Gherli (Ph.D.), under VeGIN project, Warwick Crop Centre, Wellesbourne.

2.1.9.2 GBS protocol

For the GBS protocol using next-generation sequencing (NGS), an equivalent of 300 ng and a concentration of 10-50 ng/μl was used. This was of high molecular and RNA free DNA. A restriction enzyme (ApeKI) with an insertion size of ~130 bp was used for the digestion and insertion of adaptors including the barcodes. The sequencing mode was 1 x 75 bp single-end reads, 290 million reads and ~3 million read pairs per sample.

2.2.10 Data Analysis

2.2.10.1 Morphological traits

The analysis of the morphological traits measured was performed using Excel (2016), where Mean, SD and SEM were calculated and compared between the treated and control plants performed using a Student's t-test, to test the level of significant variation was considered at $p \leq 0.05$, and 0.001. The comparison was performed within the lines only not between the lines, i.e. treated A vs control A and not vice versa. Correlation analysis between different morphological traits was also carried out to the identified level of correlation under salt stress conditions.

2.2.10.2 Physiological traits

The concentration of Na^+ , K^+ , and Ca^{2+} were determined by averaging the mean, SD, and SEM of three replicates per line, K^+/Na^+ , $\text{Ca}^{2+}/\text{Na}^+$ ratio were determined. Student's t-test analysis was conducted to determine the level of statistically significant, which were considered at $p \leq 0.05$ and 0.001. And linear regression analysis carried out between parameters to test the level of relationship in R. Principal component analysis (PCA) and Clustering analysis between the morphological and physiological traits under salt stress.

2.2.10.3 RNA-Seq Reprocessing

The reprocessing was done according to Finotello et al. (2014), the raw reads were pre-processed using some short common line tools (FASTX-Toolkit 0.0.13.2) (see Appendix VII for the scripts). The overall reads quality was inspected using the quality reports generated with FastQC. Pre-processed reads were mapped with TopHat (Kim et al., 2013) on the *B. oleracea* (To1000) (Boleracea.v2.1) genome, downloaded from the <http://brassicadb.org/brad/>. Gene coordinates file help also to map the reads spanning splices junctions (TopHat option '-G'). Reads multi-mapped were removed from the final results, together with those reads sharing less than 95% identity with reference.

In the identification of differentially expressed genes, the raw counts (BAM file) were used as input using the SeqMonk software (1.40.1) downloaded from <https://www.bioinformatics.babraham.ac.uk/projects/seqmonk/>. In the analysis workflow, SeqMonk (version 1.40.1 Babraham Institute, 2009). This is compatible and have an R language inbuilt, and pipeline for BAM files processing, generated from TopHat and can run simultaneous analyses for DSeq2 and EdgeR (v.3.8.6) Bioconductor packages were used. The software was set up to process the BAM files, and map to the sequence of *B. oleracea* genome (To1000) (Boleracea.v2.1) and scores for quality probe set at 50%. The

probes were filtered by using this inbuilt system and via the use of quality score filters, DESeq2 pipeline and EdgeR statistical analyses were conducted to obtain the differentially expressed genes using a FDR set at 0.05 for significant genes.

Table 2.5: List of membrane ion transporters identified from RNA-Seq that show differential expression 24 hour post salt shock in *B. oleracea* DH lines

Gene stable ID	Transcript ID	Chr.	start (bp)	end (bp)	Strand	Gene name	Gene description
Bo1g022080	Bo1g022080.1	C1	8072815	8076088	1	KT	Potassium transporter
Bo1g158860	Bo1g158860.1	C1	43713959	43714450	-1	V-type G	V-type proton ATPase subunit G
Bo2g024320	Bo2g024320.1	C2	6673393	6676356	-1	V-type a	V-type proton ATPase subunit a
Bo4g012670	Bo4g012670.1	C4	1353531	1360063	1	KT 9	Potassium transporter 9
Bo4g039050	Bo4g039050.1	C4	8527051	8530274	1	KUP11	K ⁺ uptake permease 11
Bo4g145930	Bo4g145930.1	C4	39931384	39935961	1	V-type a	V-type proton ATPase subunit a
Bo5g131740	Bo5g131740.1	C5	40620196	40622124	1	CAX3	cation exchanger 3
Bo8g030800	Bo8g030800.1	C8	10024031	10027008	1	V-CLC	Voltage-gated chloride channel family protein
Bo9g003910	Bo9g003910.1	C9	514674	519143	1	ECA2	ER-type Ca ²⁺ -ATPase 2
Bo9g010200	Bo9g010200.1	C9	2940449	2943704	1	NHX1	Na ⁺ /H ⁺ antiporter

* **Note:** the table shows the transcript ID as it correspond to *B. oleracea* C genome (To1000), their chromosomes (Chr.), start and end nucleotide sequence positions in each chromosome, chromosome strand, a gene name of each transcripts and gene description using conventional names.

2.2.10.4 qPCR data

Samples were run and analysis conducted using a Mx3005P multiplex quantitative PCR system (Agilent Stratagene). The housekeeping genes (β -Tubulin and TIP41) were used for data normalisation. Genotype-specific *Ct* values for each gene and control were calculated using baseline-corrected, ROX-normalised parameters. Three technical replicates included in each plate, and the average *Ct* value for each genotype was normalised within the plate housekeeping genes by a method of Livak and Schmittgen (2001). The average *Ct* values from the three biological replicates were analysed by Microsoft Excel (2016) to calculate the Mean, standard deviation (SD), % Coefficient of variance (%CV), SEM and Log₂ Fold change (FC) was determined for relative expression of genes. An ANOVA analysis conducted using F-test to test for significant variation between different time points.

2.2.10.5 GBS Data Sorting

The raw GBS read obtained were subjected to bioinformatic analysis and aligned to the reference genome sequence (*B. oleracea* To1000). The analysis generated, mapping rate, the total number of SNPs and sample allele frequency scored both at 5% and 10%. Sorting was done first, by selection of all variants sheet with 0.05 score, unidentified corresponding alleles after mapping were cleaned. Homozygous parental data was generated separately and DH lines with related homozygous parental scored to the parental type. **NOTE:** Sorting based on this format was to enable identify introgression, inversion, polymorphism and or genetic imprint of alleles in a different position of chromosomes that corresponded to our transcripts positions.

CHAPTER THREE

Morphological Variation in *B. oleracea* genotypes in Response to Salt Stress

3.0 Introduction

It is hypothesised that wild relative species have greater genetic diversity than their related cultivated varieties due to genetic drift, and therefore could be a source of genetic material for widening the gene pool. This would mean greater allelic diversity that can be exploited by plant breeders in order to develop improved crop species to ensure global food security for a growing world population.

This chapter investigates the morphological variation in response to salt stress in *B. oleracea* cultivated rapid cycling DHSL150, wild DFFS (S1) lines and DH lines derived from the F1-hybrids material. An attempt has been made to make these lines more available for trait screening through the development of the Diversity Fixed Foundation Set (DFFS) by the DEFRA funded VeGIN project (Pink et al., 2008; Walley et al., 2012). The formation of which was described in the introductory chapter (Chapter 1). The DHs derivatives are products of crosses between the wild S1 parent lines and a cultivated *B. oleracea* DH line (DHLS150) (see **Table 2.1**) and subsequently developed using a tissue culture (Microspore technique). The significance here is that two genetically diverse *B. oleracea* with different genetic background and genetic recombination have been pooled together to generate the DHs lines. This makes them an ideal collection to explore the genetic differences involved with the response to salt shock and to establish the associated pathways.

The chapter aims to investigate the extent of morphological variation in response to salt stress in *B. oleracea* cultivated rapid cycling DHSL150, wild DFFS (S1) lines and DH lines derived from the F1-hybrids material of the crosses between DHSL150 x main founder of S1 line (BolDFS).

The objective is to establish phenotypic differences by exposing the wild S1, DHSL150 and doubled haploid *B. oleracea* genotypes to salt shock stress.

This chapter will present the results of the two consecutive but separate salt stress screening experiments carried out in 2015 and 2017. The two results will be presented simultaneously to enable comparison between observed morphological variation.

3.1 Results

3.1.1 First Salt Stress Screening

3.1.1.1 Plant Height

Generally, plants response to salt stress by adapting some morphological and physiological response thereby causing significant changes in their different tissues, organs and nutrients requirements. Therefore, an effort was made to monitor plant growth was by measuring the plant height on weekly basis for three weeks after the salt stress was induced. **Figure 3.1**, presents some of the observed morphological variation in plant height among different *B. oleracea* genotypes.

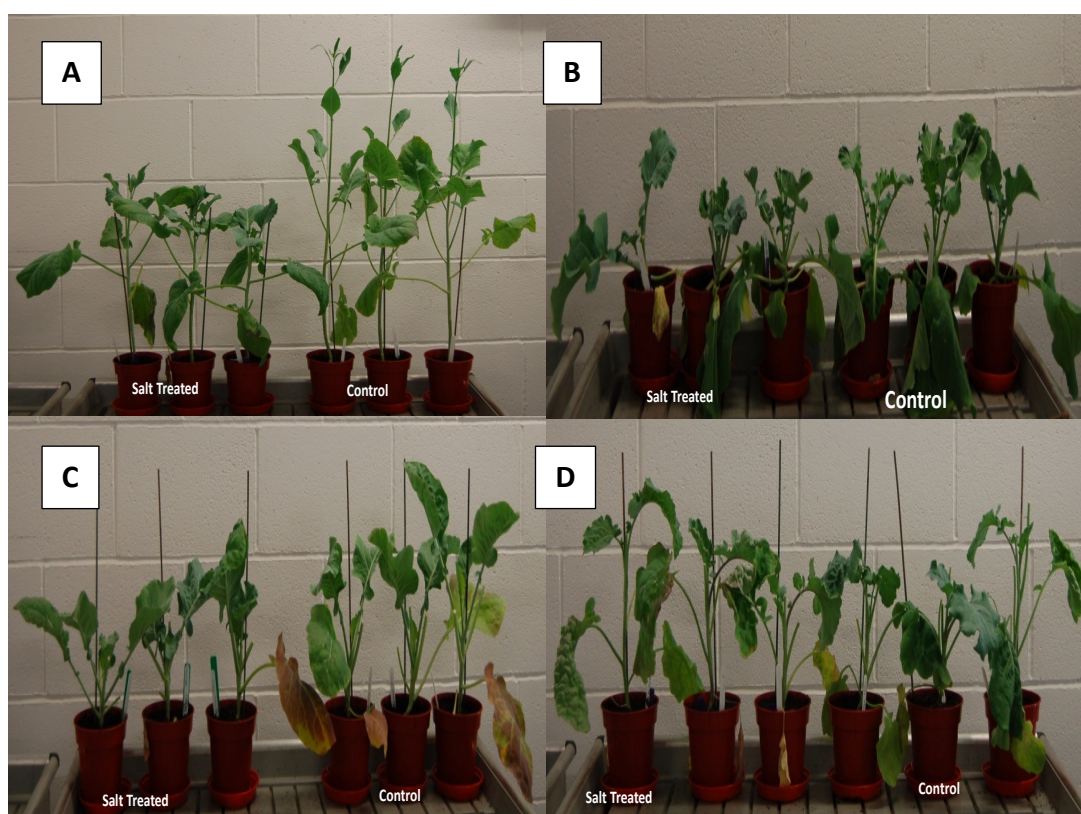


Figure 3.1: The effects of salt stress on growth of some *B. oleracea* genotypes 21 days after salt treatment from left to right: DH lines; **A)** C10121-DH; **B)** *B. oleracea* (C07079A-S1); **C)** C13013-DH & **D)** *B. oleracea* (C07060-S1).

The result presented in (**Figure 3.2a**) shows a degree of variation between and within the lines in different traits. The average growth difference between control and treatment *B. oleracea* genotypes (salt treated – untreated control), which was converted into a percent (%) and used to describe an increase or decrease in plant growth in relation to the untreated control lines. A comparison between lines was only used to describe the effects of salt stress observed between parent lines; DHSL150 and wild S1 with DH lines. The percent growth difference between control and treated *B. oleracea* genotypes showed variation between genotypes as observed in C13013-DH (-1.48%), and its related wild S1 *B. hilarionis* line (C07019-S1) (-14.96%) and DHSL150 (-11.74%). This difference between the parental lines and DH lines was also observed in DHSL150 (-11.74%), *B. bourgaei*-S1 (C07007) (-5.15%), and its two related DH lines; C10025-DH and C10027-DH which showed percent difference of between -5.79% to -11.24% respectively compared to the control plant. As presented in (**Figure 3.2a**), the cultivated line *B. oleracea* (DHSL150), the DH lines; C10025-DH and C10027-DH have shown a significant reduction in plant growth in response to salt treatment ($p < 0.05$ & $p < 0.001$). This shows that the cultivated line (DHSL150) and doubled haploids lines; C10025-DH and C10027-DH were more affected in plant growth than their related wildtype S1 *B. bourgaei*-S1 (C07007), which showed an improved growth compared to the untreated control plant. This indicates a significant morphological response between the parent DHSL150 line, the related wild S1 (C07007), and derived doubled haploid C10025-DH and C10027-DH lines under salt stress. This variation in morphological response could be due to their genetic differences / or allelic recombination effects, which have shown to influence genotype-environment interactions.

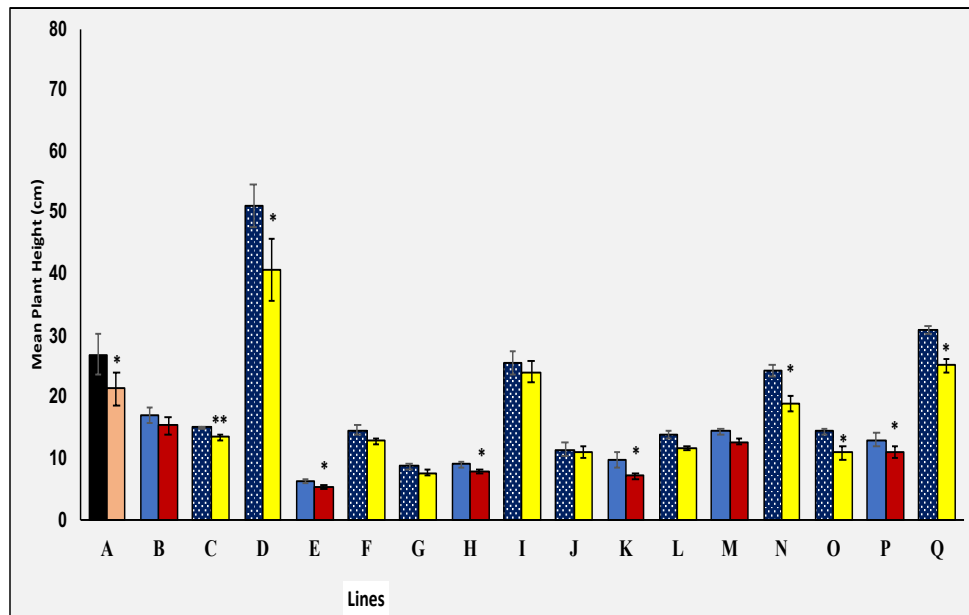


Figure 3.2a : Morphological variation in average plant growth in response to salt stress in *B. oleracea* genotypes. **Note:** A = the main founder rapid cycling line (DHSL150), B = *B. bourgaei* (C07007-S1), C = C10025-DH; D = C10027-DH; E = *B. oleracea*-S1(C07060); F = C10125-DH; G = C10128-DH; H = *B. incana*-S1 (C07094); I = C13012-DH; J = C13013-DH; K = *B. hilarionis*-S1 (C07019); L = C13001-DH; M = *B. oleracea*-S1 (C07069); N = C10132-DH; O = C10139-DH; P = *B. oleracea*-S1 (C07079A); and Q = C10121-DH. Data represent mean (n = 6) and error bars show the standard error of the mean (SEM). Significant different by comparing the mean of untreated control plant vs treated determine using **paired one-tailed** Students t-test as indicated by an asterisk (* = $p < 0.05$; ** = 0.001). Different colours represent; black = control DHSL150; light brown = treated DHSL150, dark red = treated wild S1 lines, blue = control wild S1s, yellow = treated DH lines and navy blue = control DH lines respectively. **Note:** the DH lines are placed next to their related wild S1 lines for easy follow-up while the DHSL150 remain the main parental line.

Also observed from the result (**Figure 3.2a**) was an improved growth by the DH lines C10312-DH and C13013-DH when compared against their untreated control, although this was not statistically significant ($p > 0.05$). The cultivated line DHSL150 and wild S1 (C07094-S1) showed a significant reduction in growth as compared to their untreated control lines ($p < 0.05$). In addition, this could indicate intraspecific variation between DH lines that shared a common origin, for example, C10125-DH that showed significant growth reduction as compared to the untreated control line ($p < 0.05$) and C10128-DH which showed non-significant difference to the control line ($p < 0.05$). The general observation from the result was that three DH lines; C10312-DH; C13013-DH and C10128-DH have shown an improved growth as against the control untreated lines. The same observation was made in two of the wild S1 lines; *B. bourgaei* (C07007-S1), and *B. oleracea*-S1 (C07069). The DHSL150 line has shown to be affected by salt stress that led to its significant reduction in plant height ($p < 0.05$). This result indicates morphological effects of salt stress on growth of different *B. oleracea* genotypes as compared to the untreated plants. Their response due to salt stress conditions observed could be attributed to the differences in genetic background. A good mapping population could be derived from these lines that show different genetic background for plant breeding in salt tolerant.

Furthermore, in quest to study more on the effects of salt stress on *B. oleracea* genotypes, a second salt stress experiment was carried out using the same *B. oleracea* (see **Table 2.1b**) between May-August 2017. This aims to further compare the significant variation in salt stress effects already observed between lines. As described in Chapter 2, the second salt stress experiment exposed the plants to salt treatment twice i.e., at week six (6wk) and at week eight (8wk) of plant growth (i.e., two-weeks intervals). The result (**Figure 3.2b**), shows that plant height was affected with a significant reduction observed in two of the DH

lines; C10121-DH and C13001 ($p < 0.05$). This observation corroborated with initial salt stress screening in these DH lines, which showed a reduction in plant growth as compared to the control untreated lines. Also, a reduction in plant growth was observed in the parent lines, the cultivated DHSL150, and wild related S1 lines; *B. oleracea* genotypes (C07060 & C07079A) respectively ($p < 0.05$). C100025-DH showed a non-significant growth reduction in the second screening as compared to initial salt screening that revealed a significant reduction compared to the untreated control (see **Figure 3.2a**). This observation was attributed to the fact that in the second salt stress experiment plants were exposed twice to salt treatment in a two-weeks interval, which could suggest more enhanced response from the earlier exposure of salt treatment might cause. A comparative analysis conducted to compare the effects of salt stress on plant growth between the two experiments showed no significant difference between the treated lines ($F = 0.283$).

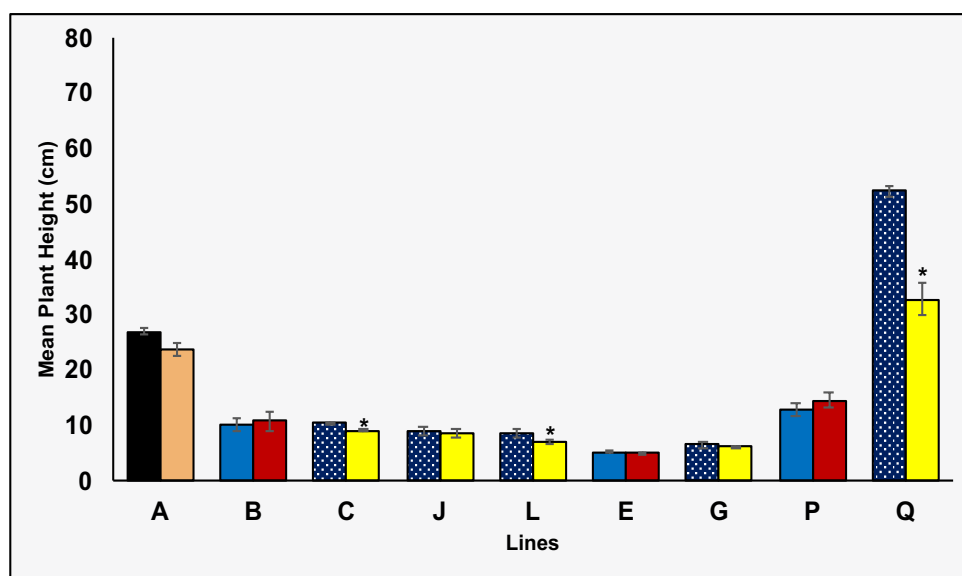


Figure 3.2b : Morphological variation in average plant growth in response to salt stress in *B. oleracea* genotypes. **Note:** **A** = the main founder rapid cycling line (DHLS150), **B** = *B. bourgaei* (C07007-S1), **C** = C10025-DH; **E** = *B. oleracea*-S1 (C07060); **G** = C10128-DH; **J** = C13013-DH; **L** = C13001-DH; **P** = *B. oleracea*-S1 (C07079A); and **Q** = C10121-DH. Data represent mean (n = 3) and error bars show the standard error of the mean (SEM). Significant different by comparing the mean of untreated control plant vs treated determine using **paired one-tailed** Students t-test as indicated by an asterisk (* = $p < 0.05$; ** = 0.001). Different colours represent; black = control DHSL150; light brown = treated DHSL150, dark red = treated wild S1 lines, blue = untreated control wild S1s, yellow = treated DH lines and navy blue = untreated control DH lines respectively.

3.1.1.2 Effects of Salt Stress on Plant's Biomass Accumulation

Salt stress affect the accumulation of biomass in plants, therefore, an increase in plant's biomass accumulation could be attributed to resilience to salt stress. On this note, two indicators of biomass accumulation in plants i.e., plant fresh and dry weights, were measured at the end of the experiment period that lasted for nine weeks (9 weeks). From the two results obtained, it was observed that plant fresh weight decreased as a result of salt shock stress (**Figure 3.3**). Although there are morphological differences between the doubled haploids and wildtype S1, some relative inhibition of biomass accumulation was observed. The wildtype S1 parental, the founder, rapid cyclic line (DHLS150) and doubled haploids lines have their fresh weight decreased with an average percent between -2.67% in *B. bourgaei*-S1(C07007) to highest -18.99% observed in C13013-DH. This indicates a clear variation between *B. oleracea* genotypes. An increase in plant fresh weight under salinity conditions could be a good indicator for salt tolerance as it indicates growth. The wild S1 lines showed a relative improvement in fresh weight as compared to the untreated control lines (**Figure 3.3**). The doubled haploids lines response more similar to the founder line (DHLS150) an effects attributed to allelic introgression. As expected both parental lines; i.e., the cultivated founder line and wild S1 lines have shown a reduction in plant fresh weight. The observed average percent decreased was between -20.14% in the main founder line (DHLS150), -2.67% to -21.87% in the wild S1 lines, and while the DH lines reduced by -12.42% and 18.99% respectively.

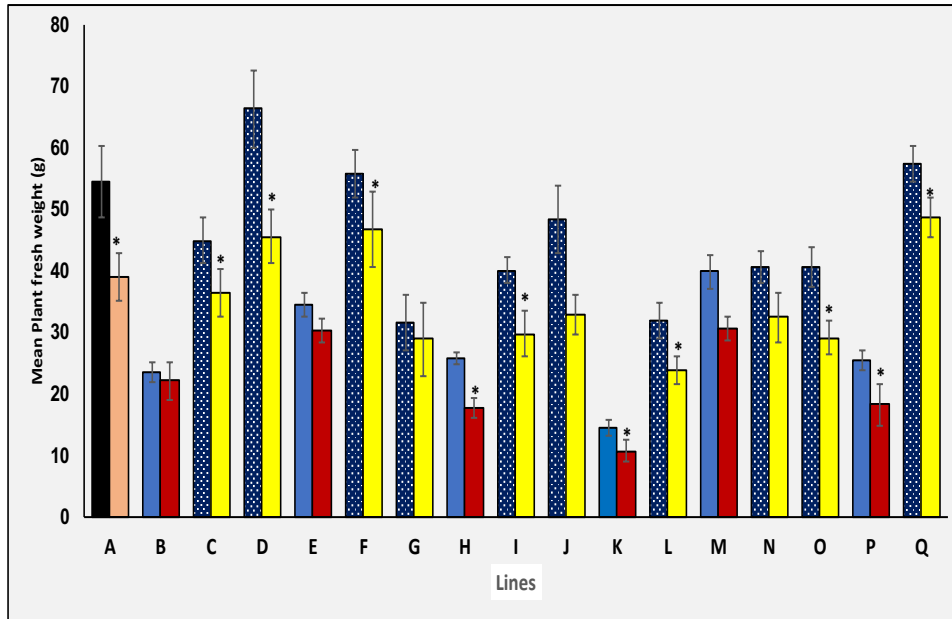


Figure 3.3: Plant fresh weight 14 days after receiving salt treatment *B. oleracea* genotypes. **Note:** **A** = the main founder rapid cycling line (DHSL150), **B** = *B. bourgaei* (C07007-S1), **C** = C10025-DH; **D** = C10027-DH; **E** = *B. oleracea*-S1(C07060); **F** = C10125-DH; **G** = C10128-DH; **H** = *B. incana*-S1 (C07094); **I** = C13012-DH; **J** = C13013-DH; **K** = *B. hilarionis*-S1 (C07019); **L** = C13001-DH; **M** = *B. oleracea*-S1 (C07069); **N** = C10132-DH; **O** = C10139-DH; **P** = *B. oleracea*-S1 (C07079A); and **Q** = C10121-DH. Data represent mean (n = 6) and error bars show the standard error of the mean (SEM). Significant different by comparing the mean of untreated control plant vs treated determine using **paired one-tailed** Students t-test as indicated by an asterisk (* = $p < 0.05$; ** = 0.001). Different colours represent; black = control DHSL150; light brown = treated DHSL150, dark red = treated wild S1 lines, blue = control wild S1s, yellow = treated DH lines and navy blue = control DH lines respectively. **Note:** the DH lines are placed next to their related wild S1 lines for easy follow-up while the DHSL150 is the main founder line.

The result of analysis on plant dry weight showed to be reduced in all the *B. oleracea* genotypes in response salt stress with a statistically significant reduction in *B. bourgaei*-S1(C07007) and *B. hilarionis*-S1 (C07019) ($p < 0.05$) (**Figure 3.4**). The doubled haploid lines; C13012-DH, C13013-DH, and C10128-DH showed to be affected by the imposed salt stress, which was non-significant as compared to the untreated control ($p > 0.05$). The calculated average percent difference indicates that plant dry weight has shown an increased by 0.12% in C10128-DH however, this was non-significant as compared to the control ($p > 0.05$). This particular doubled haploid line has recorded a non-significant reduction in the measured parameters; plant height, plant fresh weight and dry weight as compared to the

untreated control line thus indicating its resilient to salt stress as compared to the control untreated plant (**Figure 3.4**). Also, the average percent reduction observed in other DH lines was ranged between -28.52% to -12.42%, wild S1 lines -32.92% to -2.15% and finally, the cultivated founder line DHLS150 -20.14%. This analysis indicates differences between the parental lines and derived doubled haploid (DH) lines which could be due to genetics and allelic introgressions in the DH lines.

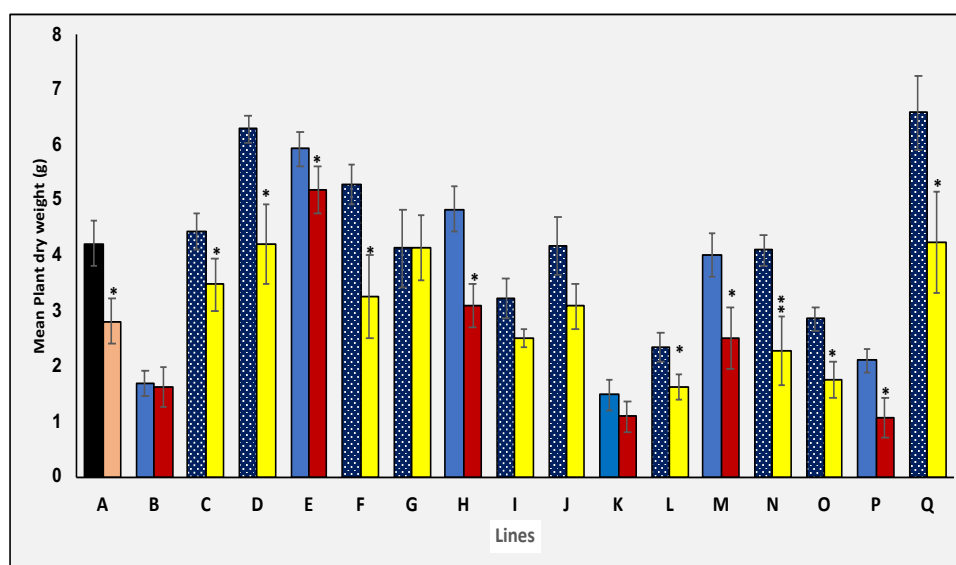


Figure 3.4: Variation in whole plant dry weight 14 days after receiving salt treatment in *B. oleracea* genotypes and oven dried at 80⁰ C for 12 hr. **Note:** **A** = the main founder rapid cycling line (DHLS150), **B** = *B. bourgaei* (C07007-S1), **C** = C10025-DH; **D** = C10027-DH; **E** = *B. oleracea*-S1(C07060); **F** = C10125-DH; **G** = C10128-DH; **H** = *B. incana*-S1 (C07094); **I** = C13012-DH; **J** = C13013-DH; **K** = *B. hilarionis*-S1 (C07019); **L** = C13001-DH; **M** = *B. oleracea*-S1 (C07069); **N** = C10132-DH; **O** = C10139-DH; **P** = *B. oleracea*-S1 (C07079A); and **Q** = C10121-DH. Data represent mean (n = 6) and error bars show the standard error of the mean (SEM). Significant different by comparing the mean of untreated control plant vs treated determine using **paired one-tailed** Students t-test as indicated by an asterisk (* = p < 0.05; ** = 0.001). Different colours represent; black = control DHSL150; light brown = treated DHSL150, dark red = treated wild S1 lines, blue = control wild S1, yellow = treated DH lines and navy blue = control DH lines respectively. **Note:** the DH lines are placed next to their related wild S1 lines for easy follow-up while the DHSL150 is the main founder line.

3.1.1.3 Effects of Salt Stress on Leaf Morphology

Morphology of the leaves differed between the *B. oleracea* genotypes, the wild S1 parental lines, cultivated founder line (DHLS150) and doubled haploid lines (**Figure 3.5**). Leaf fresh and dry weights (**Figure 3.6a & 3.6b**) showed a non-significant reduction except in *B. oleracea*-S1(C07094 and C07079A) and a DH line C13001-DH which showed a significant reduction as compared to the untreated control plants ($p < 0.05$). Contrary, an improved leaf fresh weight was observed in C10121-DH in response to salt stress as compared to the untreated line. Generally, the parental lines have shown an average percent reduction in leaf fresh weight of between -22.85% and -16.79% in wild S1 lines. While a percent increase in leaf fresh weight of 17.55% was observed in C10121-DH line respectively. This differences in leaf fresh between the *B. oleracea* lines could be due to interspecific differences / possible allelic effects that could influenced their internal mechanisms in response to any environmental stress, in this context the salt stress induced.

Contrary to what was observed in 2015 salt stress screening, *B. oleracea* genotypes showed an improved leaf fresh weight (**Figure 3.6b**) in all the genotypes in 2017 salt screening, with significant increase observed in the cultivated rapid cycling DHSL150 ($p < 0.05$) suggesting the plant's ability to respond differently after being exposed to salt treatment twice. As presented in **Figure 3.5**, different lines were observed to have developed shiny, thicker leaf surfaces, thicker petioles, and succulent leaves further suggesting improved water uptake, more photosynthesis, and more growth. Indications that suggests further changes in plant's biochemical and physiological mechanisms. This could also be used to explain the improved plant growth earlier reported in **Figure 3.2b**, where lines showed to have non-significant growth difference as compared to the untreated control lines.



Figure 3.5: Morphological variation on leaf surface area on treated *B. oleracea* species 14 days post-treatment. **a)** C10121-DH **b)** C13001-DH **c)** C07079A-S1 **d)** C07060-S1 **e)** C07007-S1 and **f)** C10128-DH. The pink tag represents treated lines and blue tag represents control. A Canon camera was set and stationed at 50 cm position from the base and set at candescent light (2017).

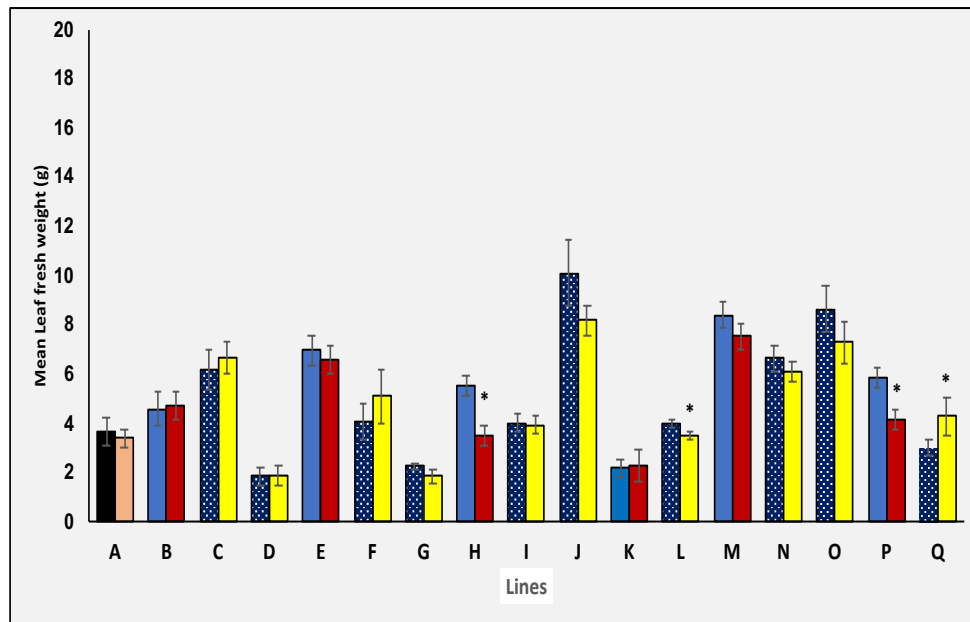


Figure 3.6a: Average Leaf fresh weight 14 days after receiving salt treatment. **Note:** A = the main founder rapid cycling line (DHSL150), B = *B. bourgaei* (C07007-S1), C = C10025-DH; D = C10027-DH; E = *B. oleracea*-S1(C07060); F = C10125-DH; G = C10128-DH; H = *B. incana*-S1 (C07094); I = C13012-DH; J = C13013-DH; K = *B. hilarionis*-S1 (C07019); L = C13001-DH; M = *B. oleracea*-S1 (C07069); N = C10132-DH; O = C10139-DH; P = *B. oleracea*-S1 (C07079A); and Q = C10121-DH. Data represent mean (n = 6) and error bars show the standard error of the mean (SEM). Significant different by comparing the mean of untreated control plant vs treated determine using **paired one-tailed** Students t-test as indicated by an asterisk (* = $p < 0.05$; ** = 0.001). Different colours represent; black = control DHSL150; light brown = treated DHSL150, dark red = treated wild S1 lines, blue = control wild S1, yellow = treated DH lines and navy blue = control DH lines respectively. **Note:** the DH lines are placed next to their related wild S1 lines for easy follow-up while the DHSL150 is the main founder line.

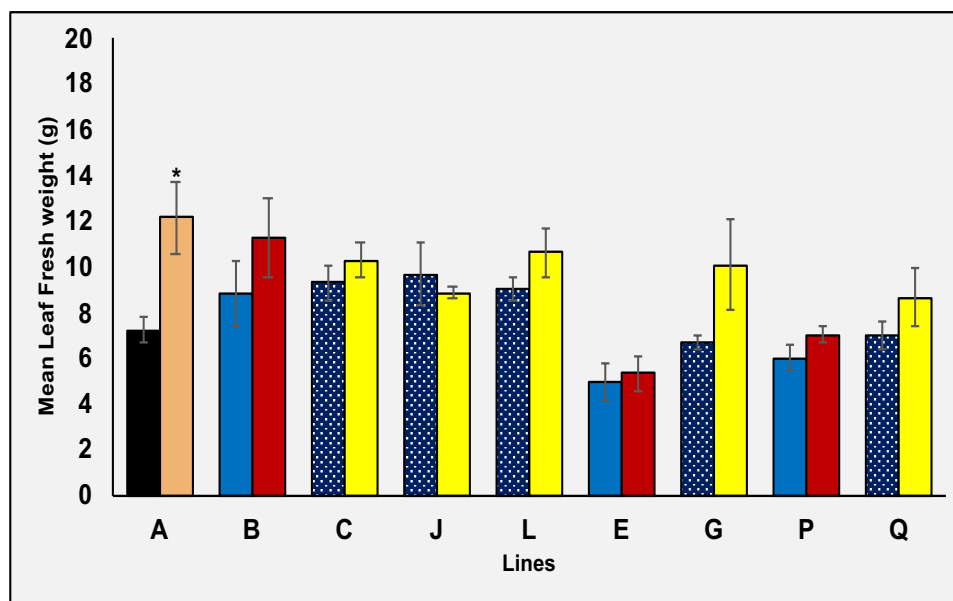


Figure 3.6b: Leaf fresh weight 21 days after receiving salt treatment in *B. oleracea* genotypes. **Note:** **A** = the main founder rapid cycling line (DHSL150), **B** = *B. bourgaei* (C07007-S1), **C** = C10025-DH; **E** = *B. oleracea*-S1 (C07060); **G** = C10128-DH; **J** = C13013-DH; **L** = C13001-DH; **P** = *B. oleracea*-S1 (C07079A); and **Q** = C10121-DH. Data represent mean (n = 3) and error bars show the standard error of the mean (SEM). Significant different by comparing the mean of untreated control plant vs treated determine using **paired one-tailed** Students t-test as indicated by an asterisk (* = $p < 0.05$; ** = 0.001). Different colours represent; black = control DHSL150; light brown = treated DHSL150, dark red = treated wild S1 lines, blue = untreated control wild S1s, yellow = treated DH lines and navy blue = untreated control DH lines respectively.

Leaf dry weight in 2017 salt screening showed a significant increase as compared to 2015 screening, where few lines indicate an increase (**Figure 3.7a**). A specific increase was observed in the cultivated founder rapid cycling DHSL150 parent line ($p < 0.05$) in both the screening (**Figure 3.7a & Figure 3.7b**) and a non-significant increase in the DH line C13013-DH, which recorded a decrease in leaf fresh weight (**Figure 3.7b**). The reasons for the improved leaf dry weight observed could be due to possible accumulated salt that causes changes in plant's leaf morphology.

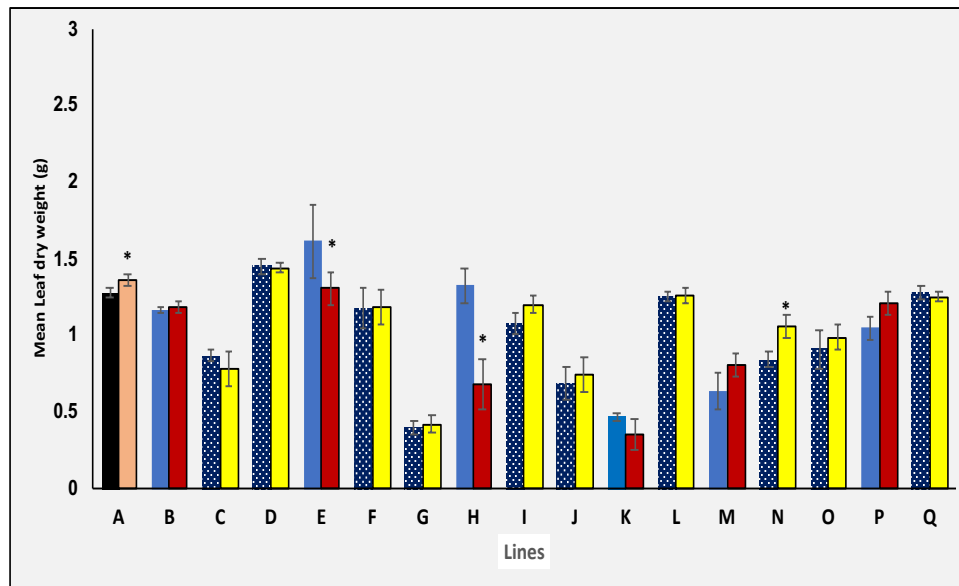


Figure 3.7a: Average Leaf dry weight 14 days after receiving salt treatment and oven dried at 80⁰ C for 12 hr. **Note:** A = the main founder rapid cycling line (DHSL150), B = *B. bourgaei* (C07007-S1), C = C10025-DH; D = C10027-DH; E = *B. oleracea*-S1(C07060); F = C10125-DH; G = C10128-DH; H = *B. incana*-S1 (C07094); I = C13012-DH; J = C13013-DH; K = *B. hiliarionis*-S1 (C07019); L = C13001-DH; M = *B. oleracea*-S1 (C07069); N = C10132-DH; O = C10139-DH; P = *B. oleracea*-S1 (C07079A); and Q = C10121-DH. Data represent mean (n = 6) and error bars show the standard error of the mean (SEM). Significant different by comparing the mean of untreated control plant vs treated determine using **paired one-tailed** Students t-test as indicated by an asterisk (* = p < 0.05; ** = 0.001). Different colours represent; black = control DHSL150; light brown = treated DHSL150, dark red = treated wild S1 lines, blue = control wild S1, yellow = treated DH lines and navy blue = control DH lines respectively. **Note:** the DH lines are placed next to their related wild S1 lines for easy follow-up while the DHSL150 is the main founder line.

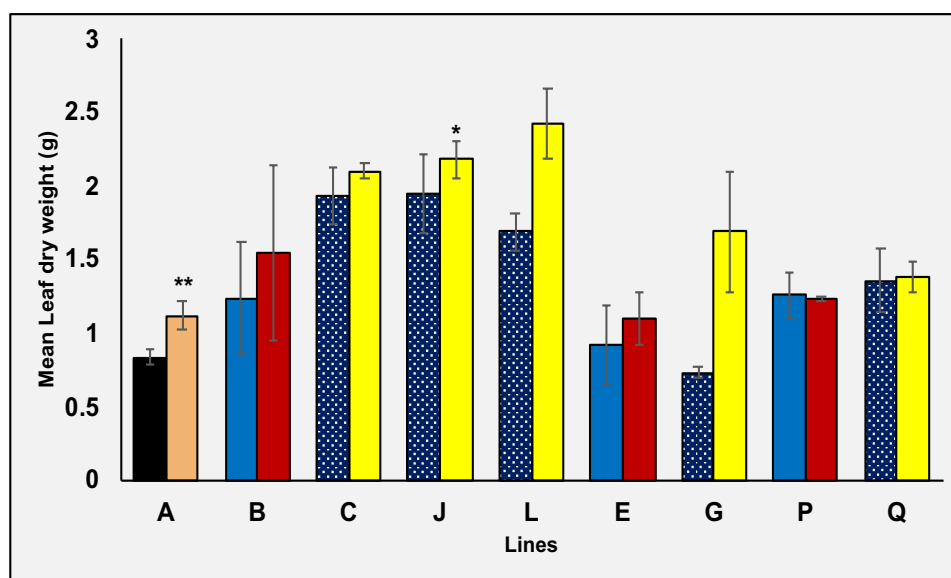


Figure 3.7b: Average Leaf dry weight 21 days after receiving salt treatment and oven dried at 80⁰ C for 12 hr. **Note:** **A** = the main founder rapid cycling line (DHSL150), **B** = *B. bourgaei* (C07007-S1), **C** = C10025-DH; **E** = *B. oleracea*-S1 (C07060); **G** = C10128-DH; **J** = C13013-DH; **L** = C13001-DH; **P** = *B. oleracea*-S1 (C07079A); and **Q** = C10121-DH. Data represent mean (n = 3) and error bars show the standard error of the mean (SEM). Significant different by comparing the mean of untreated control plant vs treated determine using **paired one-tailed** Students t-test as indicated by an asterisk (* = p < 0.05; ** = 0.001). Different colours represent; black = control DHSL150; light brown = treated DHSL150, dark red = treated wild S1 lines, blue = untreated control wild S1s, yellow = treated DH lines and navy blue = untreated control DH lines respectively.

3.1.1.4 Leaf Surface Area

The result obtained and presented in **Figure 3.8a**, shows that cultivated founder line (DHLS150) had a reduced leaf surface area ($p < 0.05$) compared against the untreated control. This was also observed in some of the wild S1 parent lines such as genotypes (C07094, C07060 and C07079A), which are all *B. oleracea*-S1 genotypes, and one of the *B. hilarionis*-S1 (C07019) genotype. This indicates that the effects are not genotype specific. There is also similarities between the DH lines that showed a significant reduction in leaf surface areas such as C13001-DH and C10125-DH. In addition, response similarity between *B. bourgaei*-S1(C07007) and its two related doubled haploids lines; C10025-DH and C10027-DH (**Figure 3.8a**) could suggests good heredity transfer from the wild species. A similar observation was also made between *B. oleracea*-S1(C07060) and the cultivated founder line DHLS150, both showed a significant reduction in leaf surface area ($p < 0.05$). In contrast the derived DH line C10128-DH, showed a non-significant reduction as against the salt untreated control. Indicating interspecific variation within the same genotype.

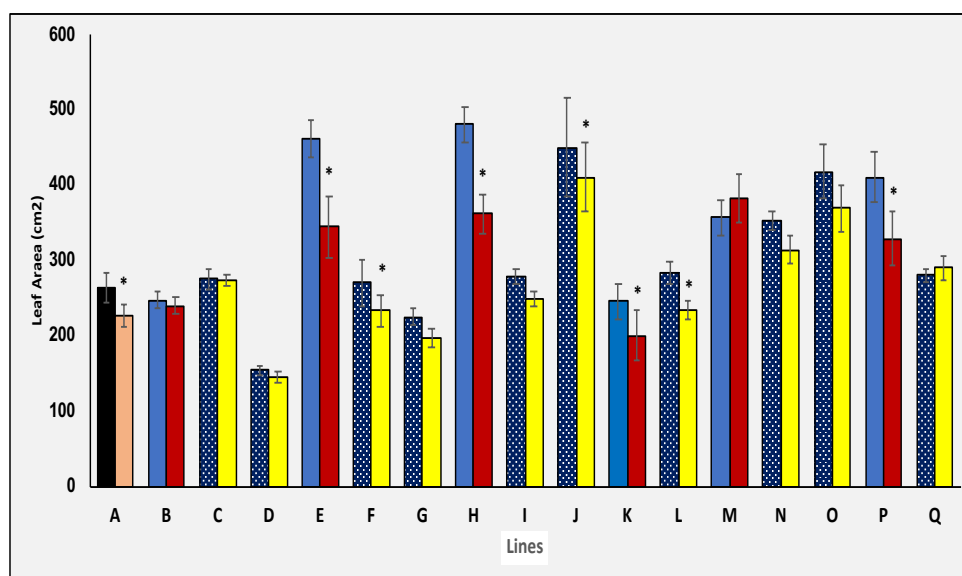


Figure 3.8a: Genotypic variation in leaf surface area 14 days after receiving salt treatment in *B. oleracea* genotypes. **Note:** A = the main founder rapid cycling line (DHSL150), B = *B. bourgaei* (C07007-S1), C = C10025-DH; D = C10027-DH; E = *B. oleracea*-S1(C07060); F = C10125-DH; G = C10128-DH; H = *B. incana*-S1 (C07094); I = C13012-DH; J = C13013-DH; K = *B. hilarionis*-S1 (C07019); L = C13001-DH; M = *B. oleracea*-S1 (C07069); N = C10132-DH; O = C10139-DH; P = *B. oleracea*-S1 (C07079A); and Q = C10121-DH. Data represent mean (n = 6) and error bars show the standard error of the mean (SEM). Significant different by comparing the mean of untreated control plant vs treated determine using **paired one-tailed** Students t-test as indicated by an asterisk (* = $p < 0.05$; ** = 0.001). Different colours represent; black = control DHSL150; light brown = treated DHSL150, dark red = treated wild S1 lines, blue = control wild S1s, yellow = treated DH lines and navy blue = control DH lines respectively. **Note:** the DH lines are placed next to their related wild S1 lines for easy follow-up while the DHSL150 is the main founder line.

Finally, leaf area measured in 2017 screening in all the genotypes has shown an improvement as compared to 2015's single dose salt screening (**Figure 3.8b**). The improved leaf surface area observed could be related to an improved leaf fresh and dry weights (**Figures 3.6b & 3.7b**). This was further attributed to a possible increase accumulation in the compatible solutes and photosynthesis. An F-test analysis conducted reveals significant variation between the two experiments ($F = 0.0067$).

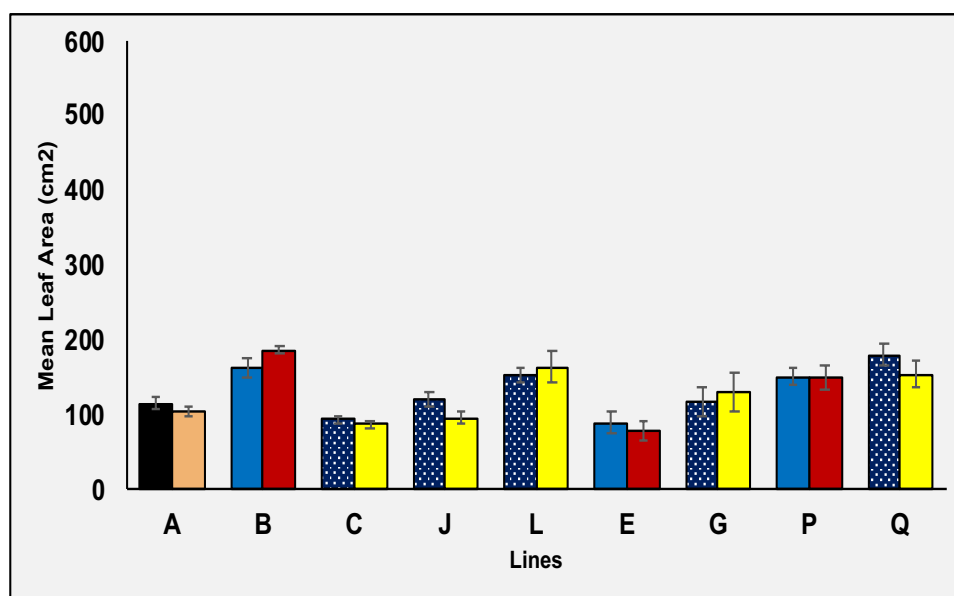


Figure 3.8b: Average Leaf surface area 21 days following salt treatment showing genotypic variation in *B. oleracea* lines. **Note:** **A** = the main founder rapid cycling line (DHSL150), **B** = *B. bourgaei* (C07007-S1), **C** = C10025-DH; **E** = *B. oleracea*-S1 (C07060); **G** = C10128-DH; **J** = C13013-DH; **L** = C13001-DH; **P** = *B. oleracea*-S1 (C07079A); and **Q** = C10121-DH. Data represent mean (n = 3) and error bars show the standard error of the mean (SEM). Significant different by comparing the mean of untreated control plant vs treated determine using **paired one-tailed** Students t-test as indicated by an asterisk (* = $p < 0.05$; ** = 0.001). Different colours represent; black = control DHSL150; light brown = treated DHSL150, dark red = treated wild S1 lines, blue = untreated control wild S1s, yellow = treated DH lines and navy blue = untreated control DH lines respectively.

3.1.1.5 Comparison of Morphological Traits

Results from correlation analysis conducted to elucidate the relationship between different phenotypic parameters measured (**Figure 3.9**), shows that plant height and plant fresh weight correlate positively in the following lines; the cultivated founder line *B. oleracea* (DHLS150), wild S1 line *B. bourgaei*-S1(C07007), and their derived doubled haploids (DHs) lines; C10025-DH and C10027-DH respectively. The correlation values were range from weak positive ($r = 0.3135$) in cultivated founder line (DHLS150) and C10025-DH ($r = 0.3530$), to strong positive correlation in wildtype S1, *B. bourgaei*-S1(C07007) ($r = 0.99987$) and derived DH line, C10027-DH ($r = 0.9979$), thus, indicating significant variation between the DH lines and parents toward salt stress response. Other parameters that showed positive correlation values presented in (**Figure 3.9**) could further suggest similar mechanisms. Contrary to earlier observation between the founder line (DHLS150) and C10025-DH that showed positive correlation against plant height and fresh weight, dry weight and plant height correlate negatively in the DH line; C10025-DH ($r = -0.4947$) while in cultivated founder DHLS150 ($r = 0.7843$) was positive. Plant height and leaf area showed variation between the parental lines and the DH line, C10025-DH ($r = -0.5294$) and the founder line (DHSL150) ($r = 0.4772$). The observed variation could be attributed to the effects of allelic variation that determine their responses to salt stress.

The DH line, C13013-DH showed positive correlation between leaf area and plant height ($r = 0.8580$), leaf area/fresh weight ($r = 0.9839$), leaf area/dry weight ($r = 0.9626$) and leaf area/leaf fresh weight ($r = 0.9875$) respectively. In addition, a clear differences between the wildtype S1 parent line; *B. incana*-S1(C07094) and their derived DH C13013-DH, could suggests better performance by the DH line in response to salt stress against the control.

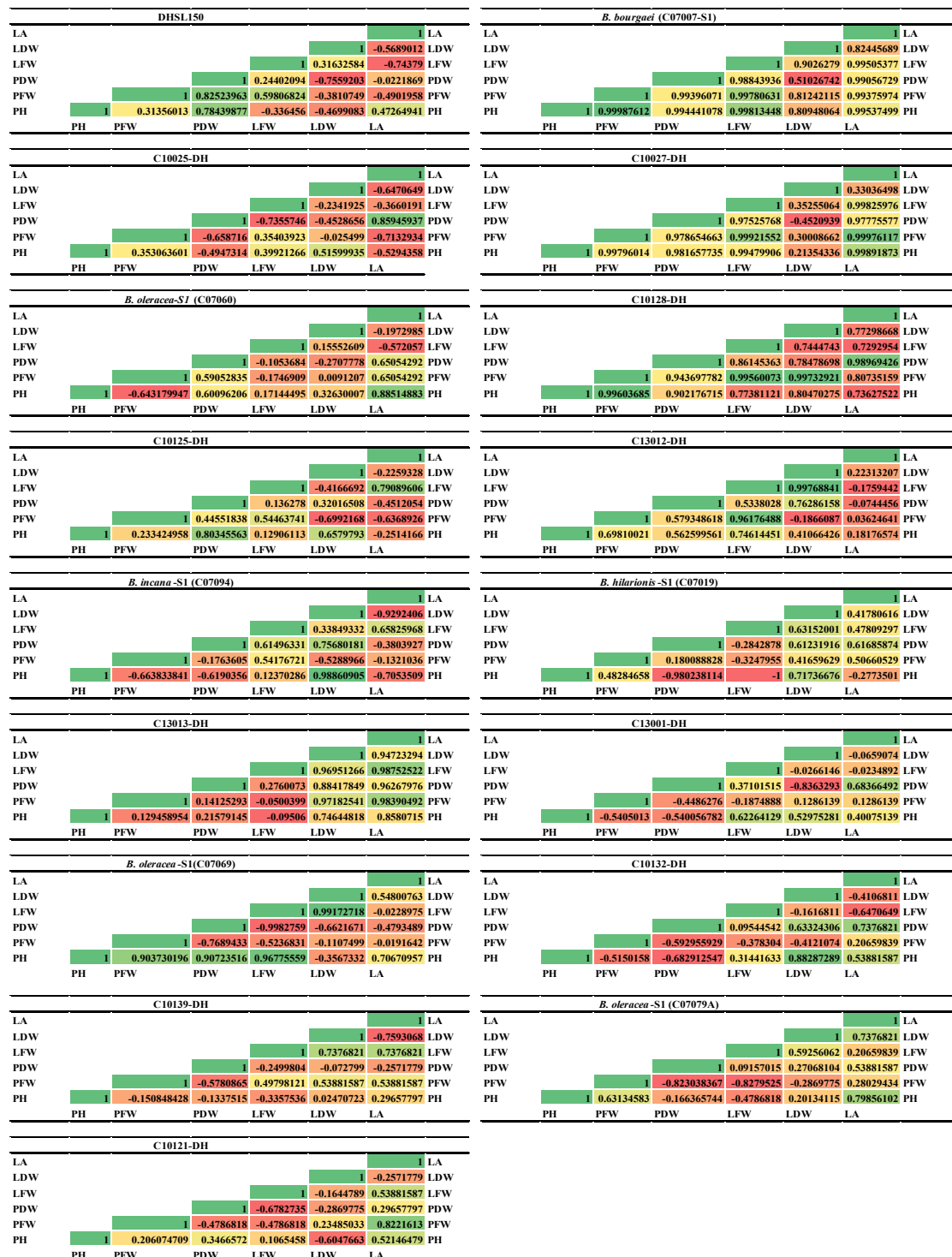


Figure 3.9: A heat map of Pearson correlation analysis between phenotypic parameters in *B. oleracea* genotypes in response to salt shock. **Colour Key:** Green = perfect correlation, light-green to yellow = good positive correlation and light-red to dark-red = negative correlation. LA = leaf area, LDW = leaf dry weight, LFW = leaf fresh weight, PDW = plant dry weight, PFW = plant fresh weight and PH = plant height.

3.2 Discussion

3.2.1 Plant Growth

The response of *B. oleracea* genotypes under salt stress conditions was investigated. Plant height was shown to be affected by salt stress in some of the *B. oleracea* genotypes indicating significant variation. More effects in plant growth were observed in parent lines such includes; the cultivated founder line, *B. oleracea* (DHLS150), wildtype S1 parent lines, *B. incana* (C07094-S1) and *B. oleracea* (C07079A-S1) showed a significant reduction as compared to the untreated control ($p < 0.05$) (**Figure 3.2a**). Also, salt stress causes a significant reduction in the growth of some of the doubled haploid lines; (C10121-DH), (C10025-DH) as compared to control. A reduction in plant growth due to salt stress has also been reported in different plant species (Essa, 2002; Cicek and Cakirlar, 2002; Li et al., 2006, Kusvuran et al., 2007; Tunçtürk et al., 2008). Salt stress has shown to provoked osmotic potential, which interfered with root cells ability to obtain the required water from the soil (Mer et al., 2000). Thereby restricting plants uptake of some dissolved mineral nutrients in the water. Thus, growth and development of plants are both inhibited due to the occurring defect in metabolism under salt stress (Mer et al., 2000). Also, researchers thought that the growth reduction due to salinity stress could be the consequences of ion accumulation through changing of membrane permeability (Cramer et al., 1985; Grieve and Fujiyama, 1987). Most crops exposed to the saline conditions showed a reduction in growth. The deleterious effects of salinity could further be suggested as a result of water stress, ion toxicities, ion imbalance, and or a combination of all these factors (Kurth et al., 1986). An improve plant growth was also observed in some of the wild S1 parent lines; *B. bourgaei* (C07007-S1) *B. oleracea*-S1 (C07060-S1) and doubled haploid (DH) lines; (C10128-DH) and (C13013-DH), which showed a non-significant reduction against the untreated control plants (**Figure 3.2a**). An improve plant growth under salinity conditions could be attributed

to the ability of the plant to adjust their water uptake system, which would further their photosynthesis and accumulation of growth hormones (Mer et al., 2000).

3.2.2 Biomass accumulation

Plant fresh and dry weights of treated *B. oleracea* genotypes were reduced following salt treatment. A significant reduction was observed in the cultivated founder line DHSL150, wild S1 parent lines; *B. oleracea* (C07060-S1, C07069-S1 and C07079A-S1), and *B. incana* (C07094-S1) and derived doubled haploid lines; (C10125-DH & C10139-DH), (C10121-DH), (C10025-DH & C10027-DH), and (C13012-DH & C13013-DH), and finally (C13001-DH) as compared to the untreated control plants. Siddiqui et al. (2009) have reported a reduction in plant fresh weight in a combined drought and salt-stressed treatments on *B. napus* genotypes but with a considerable increase in dry weight. It has been suggested that both applications of drought and salt stress significantly affect the water status of the soil; as a result, the water uptake becomes affected leading to fresh weight reduction. An increase in biomass accumulation under salt stress was also reported by Jones and Turner (1980); Munns and Weir (1981) and the studies by Jamil et al. (2007b) on radish plants (*Kyllinigia peruviana* L.), and Memon et al. (2010) on *Brassica campestris* L reported an increase in biomass accumulation in salt stress conditions.

Plant leaf, an essential organ that plays a critical role in photosynthesis, photorespiration, and transpiration for healthy growth of any plant. *B. oleracea* genotypes have shown an improved leaf fresh and dry weights. However, a significant reduction was observed in the wild S1 lines; *B. incana* (C07094-S1), *B. oleracea* (C07079A-S1) and doubled haploid lines; (C10121-DH), (C10132-DH) and (C13001-DH). The difference observed could be attributed to the effects of salinity stress and variation in plant's ability to accumulate

biomass, which is essential and pre-requisite for the accumulation of osmotic solutes (soluble carbohydrate, protein, and total free amino acids). Accordingly, plant salt tolerance could be genotype dependent and because it involves a complex mechanism thus affect water retention and synthesis of osmotically active metabolites (Azooz, 2009; Sarwat and El-Sherif, 2007).

3.2.3 Leaf surface area

An improved leaf area under abiotic stress has been described as indicator of plant growth, which could be affected by different stresses including salinity stress. The *B. oleracea* genotypes showed variation in leaf area following salt treatment as compared to the untreated control. A significant decrease in leaf area was observed in the cultivated rapid cycling lines DHSL150, *B. oleracea*-S1 (C07094-S1), *B. hilarionis*-S1 (C07019-S1), and *B. oleracea*-S1 (C07079A-S1) (Figure 3.8a). Also reduction in leaf area was observed among the DH lines; C10312-DH, and C10125-DH as against to untreated control (**Figure 3.8a**). Studies have implicated salt stress in plant leaf area reduction observed in moth bean plant (*Vigna aconitifolia* L.) (Mathur et al., 2006); sugar cane (*Beta vulgaris*) (Jamil et al., 2007). Other supporting research includes those of Zhao et al. (2007) on oat (*Avena sativa* L.); Yilmaz and Kina (2008) on *Fragaria x anassa* (L.), both found that exposure to salinity leads to a reduction in leaf area. A decrease in leaf area has been attributed to an adverse effect of excess salt on photosynthetic elements that lead to a reduction in chlorophyll content, reduced plant growth and consequently leaf area (Netonda et al., 2004). Likewise, a decrease in leaf area has been attributed to the stomatal closure due to the effect of salt stress to minimize water loss via transpiration (Alarcón et al., 2006) and finally, a study by Nima and Mahmoud (2017) reported a reduction in leaf area in *B. napus* L genotypes in response to salt stress.

Others that showed an improved leaf area, were mainly doubled haploid lines (**Figure 3.8b**) and one of the wild S1 line, *B. oleracea* (C07069-S1) as compared to the untreated control lines. An increase in leaf area was attributed to leaf expansion, a critical mechanism employed by plants to improve their leaf surface area, thus reduce the effects of accumulated salt on homeostasis, nutrients acquisition and enhanced photosynthesis (Marschner, 2002).

3.3 Summary

The chapter reported the effects of salt stress on plant's morphology in *B. oleracea* genotypes in two salt stress experiments using the same genotypes. It aims to study the morphological variation in prompt response to salt stress in *B. oleracea* genotypes. These genotypes are related genetically, where the cultivated founder rapid cycling line DHSL150 is the main parent line to DH lines and wild S1 lines representing the founder wild DFS parent lines, which were not included in the screening. The chapter also reported significant variation among *B. oleracea* genotypes in all the morphological traits measured. Indicating variability which can be linked to their recombinant genetic differences. Different parameters for salt stress resilient such as plant growth has shown to be affected most significantly as against the untreated controls in some parents; DHSL150, wild S1 lines; C07094-S1, C07019-S1, C07060-S1, C07019-S1, and C07079A-S1. This significant reduction in plant height was also reported in some DH lines; C10025-DH, C10027-DH, C13001-DH, C10125-DH, C10132-DH, C10139-DH and C10121-DH against their control. This indicates that the DH lines might have inherited the same trait from the parent lines. Also reported in this chapter are a non-significant reduction in plant height as against the control in some wild S1 and DH lines respectively. Such wild S1 are C07007-S1 and C07069-S1, and the DH lines; C13012-DH, C13013-DH, and C10128-DH. Other traits reported that show significant variability as against the salt untreated control are; whole plant fresh weight, whole dry weight, leaf fresh weight, leaf dry weight, and leaf area. Based on these traits investigated under two separate salt stress screenings, we can draw an inconclusive argument that some of the wild S1 and DH lines have shown more salt stress resilience than the cultivated founder DHSL150 as compared to the untreated control plants. However, further work is recommended to substantiate our observation.

CHAPTER FOUR

Variation in Mineral Content in *B. oleracea* genotypes in Response to Salt Stress

4.0 Introduction

Under salt stress conditions, plants are forced to regulate their water and nutrients uptake. Salt stress changes ion composition and ion concentrations within the plants (Munns and Tester 2008). An Increase in dissolved salts in the soil causes reduction in the water energy gradient thus making it more difficult for water and nutrients to shuttle through root membranes and into the plant. This is due to the fact that movement of water and solutes from soil into the plant is guided by osmotic potentials on root membranes. Essential elements that are involved include potassium (K^+), calcium (Ca^{2+}) and in salinity conditions, the presence of sodium (Na^+) ions. Potassium and calcium ions are critical and most important for the maintenance of water potential, cell turgor, and overall cell integrity. Calcium was shown to play a crucial role in the formation of intermolecular linkages, which is significant for integrity and structure of plasma membranes and also used as a second messenger in the propagation of signal down many of biological pathways (Hanson, 1984; Bush, 1995). Potassium, on the other hand, is vital in plants-water relation, maintenance of water potential and play complementation of many enzymes reactions and serve as osmoticum in cell vacuole. Sodium ions may not be that essential in most plants, especially glycophytes. Many plants have been shown to be stimulated using low Na^+ concentrations, and, sodium has been identified as being critical for halophytes. C4 and other halophytes require high levels of Na^+ for maximum growth (Flowers et al., 1977).

The response of plants to salinity has been summarised into two main phases. The shoot ion-independent response that occurs within minutes to days, and thought to be related to Na^+ sensing and signalling (Gilroy et al., 2016; Roy et al., 2014) and the ion-dependent response, which has shown to develop over a more extended period. It involves a build-up of ions in the shoot inflicting toxicity affecting mainly old leaves, causing premature senescence of leaves and ultimately reduced growth and yield (Munns and Tester, 2008).

Salt tolerance as proposed by Munns and Tester (2008) is related to the plant's ability to exclude ion to avoid being accumulated to a toxic level. Tissue tolerance often is associated with compartmentalization of toxic ions into specific tissues, cells and subcellular organelles. Shoot ion-independent tolerance, requires the maintenance of growth and water uptake in the face of Na^+ accumulation. The difference in a plant's response and tolerance to a given level of salt stress is dependent upon the concentration, composition of ions and as well as the genotype (reviewed: Thomas and Ralph 2018).

The chapter aims to study the variation in leaf mineral content in each of the *B. oleracea* genotypes that were exposed to salt stress as compared to salt untreated control. The objective is to determine the level of Na^+ , K^+ , and Ca^{2+} in the leaves of *B. oleracea* genotypes following exposure to salt stress.

4.1 Results

4.1.1 Variation in Mineral Content First Salt Stress Screening

The effects of 250 mM NaCl salt treatment on *B. oleracea* genotypes cause a significant increase in Na⁺ content twenty-four hours post-treatment (**Figure 4.1**). According to the result, the nutrients content of treated plants showed an increased Na⁺ content which could affects the K⁺ ion level. This was statistically significant ($p < 0.05$) although the Ca²⁺ level remained unchanged. A comparison between the two different time points of data collection i.e., twenty-four hours and two-week post-treatment, showed significant variation in the level of Na⁺ ion within the *B. oleracea* leaves. Two-weeks post-treatment, the Na⁺ ion levels was observed to reduce significantly across the lines and the ability to reduce it can be used as a marker for salt tolerance. The DH line, C10128-DH has shown an increase in Na⁺ level and reduction in K⁺ twenty-four post-treatment as compared to two-weeks post-treatment ($p < 0.05$) and Ca²⁺ level remain relative unchanged (**Figure 4.1**).

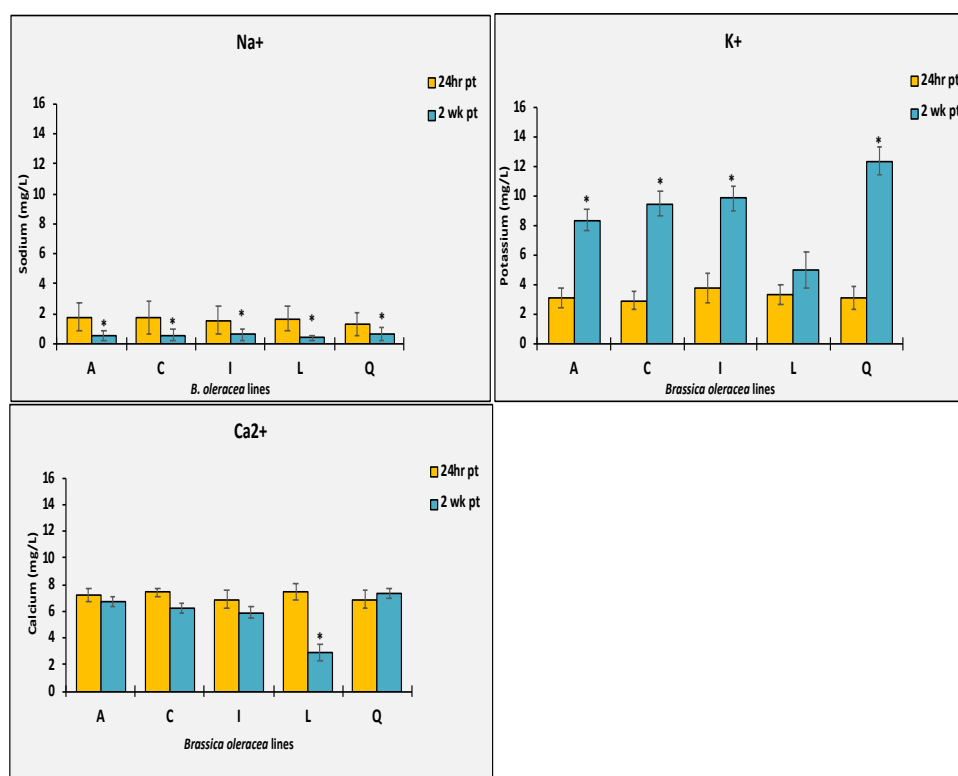


Figure 4.1: The effects of 250 mM NaCl salt treatment on mineral content, which caused a significant increase in Na⁺ ion twenty-four hours post-treatment and the ability of the plants to reduce the Na⁺ ion, improved their K⁺ while Ca²⁺ level unchanged. The values are Mean (n = 4), error bars are SEM and asterisk (* = p < 0.05; ** = p < 0.001). **Legend:** 24 hrpt = 24 hr post-treatment (**Orange colour**), 2 wkpt = 2 weeks post-treatment (**dark cyan colour**). **Note:** A = the main founder rapid cycling line (DHLS150), C = C10025-DH; I = C13001-DH; L = C10128-DH, and Q = C10121-DH. And further note: This comparisons was **only** done on the salt treated plants as no salt untreated control were included in the mineral analysis in 2015 salt screening. So, we compared mineral content in leaf 24 hr post-treatment and 2 week post-treatment.

The relationship between Na^+ ion, K^+ and Ca^{2+} were tested using a general linear model, and the regression value (R^2) was determined 24 hr post-treatment (**Figure 3.2**). The result showed Na^+ , K^+ and Ca^{2+} were positively correlated with regression values of $R^2 = 0.097$ and 0.680 respectively (**Figure 4.2**). which is weaker as compared to two-week post-treatment with regression values between ($R^2 = 0.973$ & 0.908) respectively. Na^+ interference with both K^+ and Ca^{2+} reported in some studies could be dependent upon length of exposure and could further show the efficiency of the mechanisms involved in *B. oleracea* genotypes have been compromised by excess Na^+ .

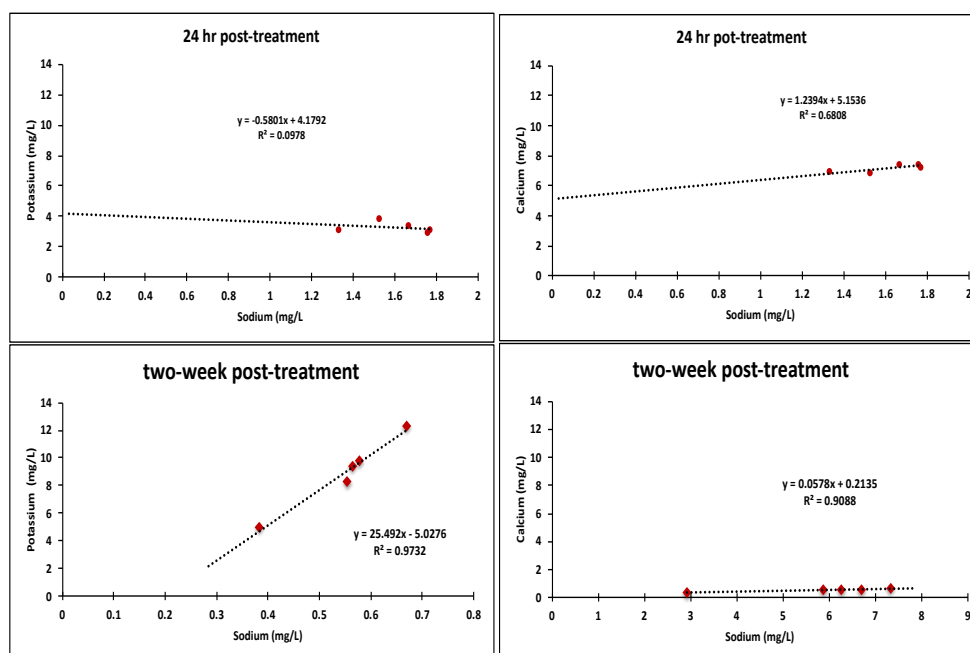


Figure 4.2: Regression analysis showing the relationship between Na^+ , K^+ , and Ca^{2+} following application of 250 mM NaCl to *B. oleracea* genotypes at 24 hr post-treatment and two-week post-treatment for five *B. oleracea* lines, i.e., DHLS150, C10025-DH; C13001-DH; C10128-DH, and C10121-DH.

It becomes evident that the *B. oleracea* genotypes in this study respond similarly averting salt accumulation and improving K^+ ion uptake while keeping Ca^{2+} ion level relatively unchanged. Ca^{2+} plays a critical role as secondary messenger, and growth enhancer factor and therefore changes in its concentration can be critical.

4.2.2 Na^+/K^+ Ratio (first salt screening)

Generally, the level of salt-tolerance or susceptibility as a result of salt treatment was determined by calculating ratios of K^+/Na^+ , Ca^{2+}/Na^+ , and K^+/Ca^{2+} by using the two-point data, and comparison carried out to determine the level of tolerant or otherwise and result presented in **Table 4.1**.

Table 4.1: Ratios of nutrient content in *B. oleracea* genotypes following Salt Treatment

Sample ID	Na (mg/L)	SEM	K (mg/L)	SEM	Ca (mg/L)	SEM	K^+/Na^+ ratio	Ca^{2+}/Na^+ ratio	K^+/Ca^{2+} ratio
DSLH 150-24hr	1.770	0.953	3.083	0.640	7.227	0.485	1.742	4.084	0.427
C10025-24hr	1.758	1.076	2.903	0.619	7.401	0.359	1.651	4.209	0.392
C10121-24hr	1.334	0.764	3.109	0.752	6.871	0.705	2.331	5.153	0.452
C10128-24hr	1.667	0.822	3.346	0.665	7.404	0.621	2.007	4.442	0.452
C13001-24rh	1.527	0.937	3.783	1.011	6.849	0.677	2.477	4.484	0.552
DSLH 150-2 wk pt	0.554	0.343	8.333	0.735	6.700	0.352	15.035	12.089	1.244
C10025-2 wk pt	0.565	0.363	9.443	0.831	6.266	0.358	16.728	11.100	1.507
C10121-2 wk pt	0.670	0.440	12.353	0.982	7.337	0.422	18.450	10.958	1.684
C10128-2 wk pt	0.384	0.190	4.988	1.231	2.921	0.641	13.002	7.614	1.708
C13001-2 wk pt	0.578	0.356	9.838	0.832	5.877	0.452	17.029	10.172	1.674

Note: 24 hr = 24 hr post-treatment and 2 wk pt = two-weeks post-treatment, red colour indicates significant reduction in K^+/Na^+ ratio and light-green to dark-green indicate an improved K^+/Na^+ ratio.

From the result, it was observed that both the founder parent line (DHLS150) and DH lines showed an improve K^+/Na^+ ratio two-weeks post-treatment, which indicates improve resilience as compared to the K^+/Na^+ 24 hr post-treatment (**Table 4.1**).

4.2.3 Variation in Mineral Content: Second Salt Stress Experiment

The experiment was repeated in 2017 by adapting same design and with same number of DH lines but in this case, the wild S1 lines were added as described in Chapter 2. This was to enable observation of greater potential physiological variability among the parent lines and DH lines. **Note:** an additional salt induction was carried out at two-weeks post first induction i.e., at week-eight of plants growth as stated in Chapter 2.

From the result, Na⁺ level increased twenty-four hours post-treatment in all the *B. oleracea* genotypes as indicated (**Figure 4.3**). This was statistical significance in the founder parental line, *B. oleracea* DHLS150, C10121-DH, C10025-DH, C13013-DH and C13001-DH ($p < 0.001$). While the wild S1 parental lines; *B. bourgaei*-S1 (C07007-S1), *AB. oleracea*-S1 (C07079A-S1), and *B. oleracea*-S1 (C07060-S1) and doubled haploid line, C10128-DH have shown a non-significant increase in the level of Na⁺ ions twenty-four hours post-treatment.

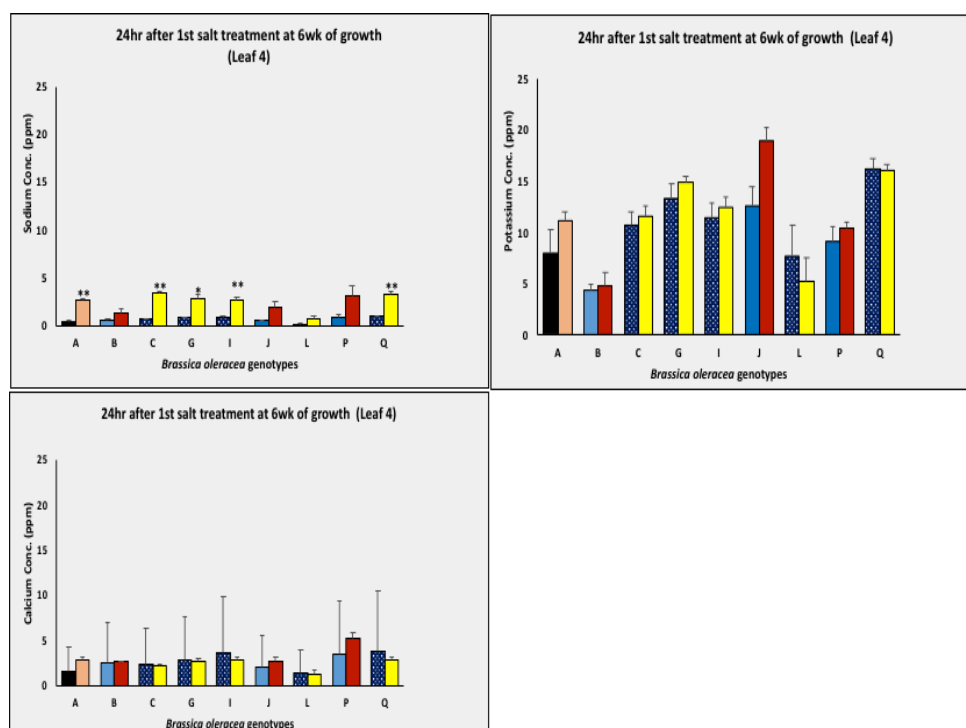


Figure 4.3: Mineral contents of *B. oleracea* genotypes twenty-four hours post-treatment. **Note:** **A** = the main founder rapid cycling line (DHLS150), **B** = *B. bourgaei* (C07007-S1), **C** = C10025-DH; **G** = C13013-DH; **I** = C13001-DH; **J** = *B. oleracea*-S1(C07060); **L** = C10128-DH; **P** = *B. oleracea*-S1 (C07079A); and **Q** = C10121-DH. Data represent mean (n = 3) and error bars show the standard error of the mean (SEM). Significant different by comparing the mean of untreated control plant vs treated determine using **paired one-tailed** Students t-test as indicated by an asterisk (* = p < 0.05; ** = 0.001). Different colours represent; black = control DHSL150; light brown = treated DHSL150, dark red = treated wild S1 lines, blue = untreated control wild S1s, yellow = treated DH lines and navy blue = untreated control DH lines respectively.

The potassium (K^+) ion level remained significantly unchanged following salt treatment 24 hr post-treatment in *B. oleracea* genotypes, which signifies that an increase in Na^+ ion level might have no effect on K^+ accumulation twenty-four hours post-treatment and calcium ion as demonstrated in **Figure 4.3**.

Two-weeks post-treatment, the result indicates a higher Na^+ level in all the salt treated *B. oleracea* genotypes as compared to untreated control (**Figure 4.4**). Although the potassium ion level between treated and control shows non-significant differences (p > 0.05), a significant reduction was observed in both the founder parent line; DHLS150 and a doubled haploid line; C13013-DH. In addition, two of the wild S1 lines; *B. bourgaei*-S1 (C07007),

and *B. oleracea*-S1(C07060) and DH line; C10128-DH were shown to retain higher potassium levels two-weeks post-treatment as compared to other lines (**Figure 4.4**)

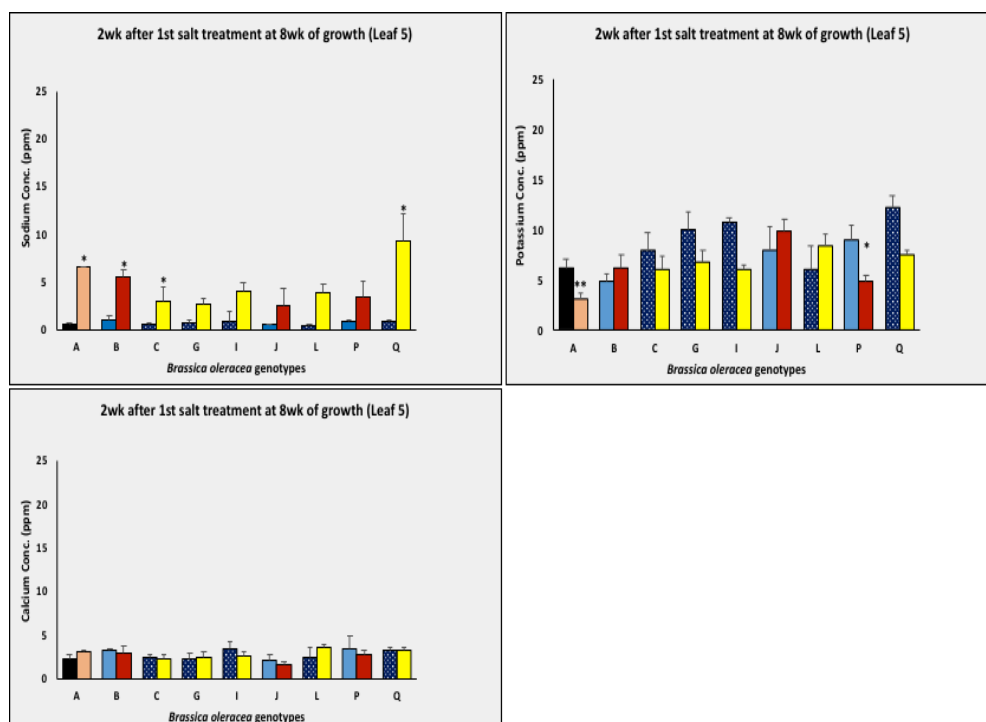


Figure 4.4: Variation in leaf mineral content of Na⁺, K⁺ and Ca²⁺ two-week post salt treatment in *B. oleracea* genotypes. **Note:** A = the main founder rapid cycling line (DHLS150), B = *B. bourgaei* (C07007-S1), C = C10025-DH; G = C13013-DH; I = C13001-DH; J = *B. oleracea*-S1(C07060); L = C10128-DH; P = *B. oleracea*-S1 (C07079A); and Q = C10121-DH. Data represent mean (n = 3) and error bars show the standard error of the mean (SEM). Significant different by comparing the mean of untreated control plant vs treated determine using **paired one-tailed** Students t-test as indicated by an asterisk (* = p < 0.05; ** = 0.001). Different colours represent; black = control DHSL150; light brown = treated DHSL150, dark red = treated wild S1 lines, blue = untreated control wild S1s, yellow = treated DH lines and navy blue = untreated control DH lines respectively.

In a bid to explore further possible physiological variation in *B. oleracea* genotypes, an additional salt treatment was carried out at week-eight of plants growth i.e., a two-weeks post earlier salt treatment (see chapter 2). Leaves samples were collected twenty-four hours post-treatment. The result obtained (**Figure 4.5**) shows higher level of Na⁺ level in all the treated DH lines and the founder DHSL150 as compared to the two other wild S1 lines; C07060-S1 and C07079A respectively. A significant increase in Na⁺ was noted in the wild S1; C07007 compared to its control. It is of interest that the K⁺ level remained significantly

higher despite excess salt. This could indicate a mechanism employed by these genotypes to counteract the Na^+ effects through maintenance of high K^+ . Calcium level remains relatively unchanged as compared between lines and untreated plants.

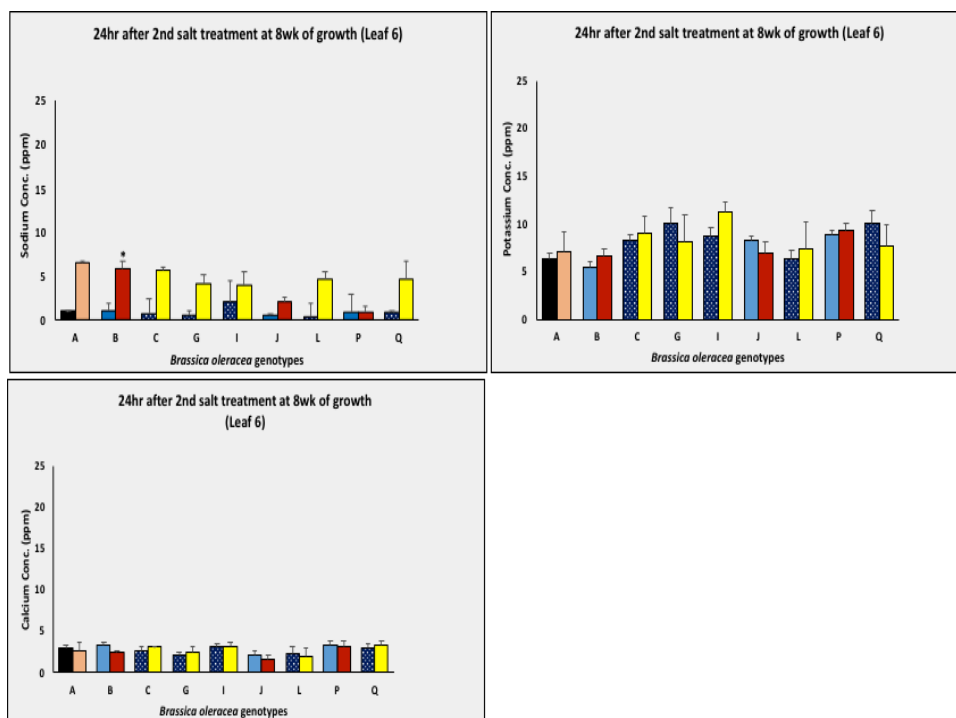


Figure 4.5: Difference in Na^+ , K^+ and Ca^{2+} level in *B. oleracea* genotypes 24 hr post-salt treatment. **Note:** A = the main founder rapid cycling line (DHSL150), B = *B. bourgaei* (C07007-S1), C = C10025-DH; G = C13013-DH; I = C13001-DH; J = *B. oleracea*-S1(C07060); L = C10128-DH; P = *B. oleracea*-S1 (C07079A); and Q = C10121-DH. Data represent mean (n = 3) and error bars show the standard error of the mean (SEM). Significant different by comparing the mean of untreated control plant vs treated determine using **paired one-tailed** Students t-test as indicated by an asterisk (* = $p < 0.05$; ** = 0.001). Different colours represent; black = control DHSL150; light brown = treated DHSL150, dark red = treated wild S1 lines, blue = untreated control wild S1s, yellow = treated DH lines and navy blue = untreated control DH lines respectively.

Two weeks treatment, the Na^+ level was significantly reduced in some DH lines as compared to the parents; DHSL150 and the wild S1 line C07007 which both retained high Na^+ concentrations within the leaves (Figure 4.6). The reduction in Na^+ level could be through compartmentalisation or exclusion processes via activation of non-selection cation channels (NSCCs) such as out-right rectifiers channels, Na^+/H^+ antiporter proteins (NHX)

otherwise known as SOS1 in the salt overly sensitive pathway that operates in the root cells of the plants.

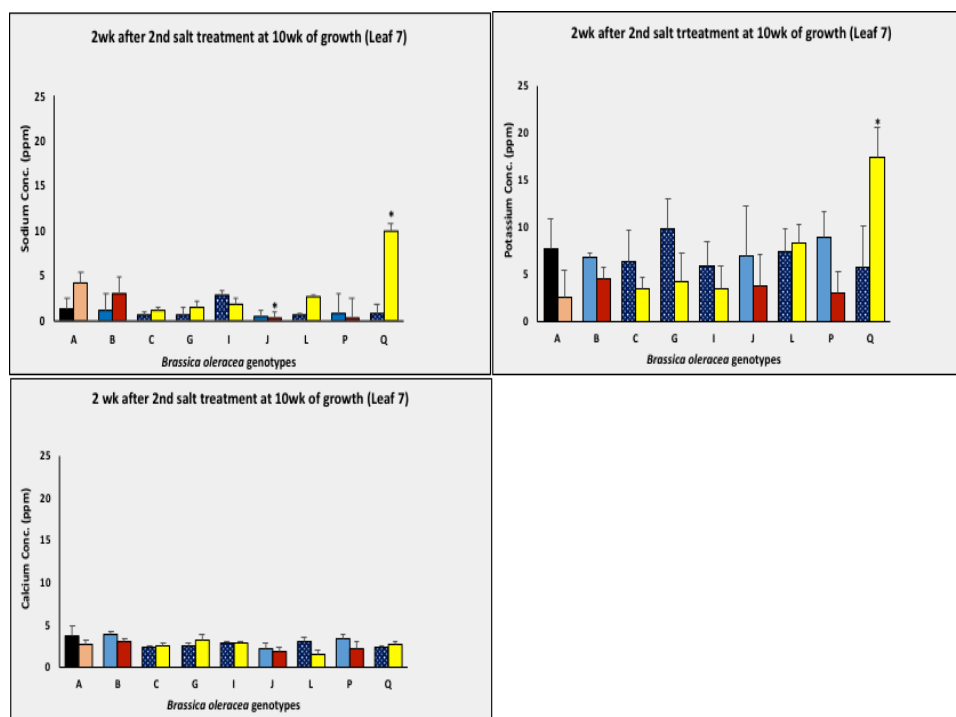


Figure 4.6: Genotypic variation in leaf content of Na⁺, K⁺ and Ca²⁺ two-weeks after salt treatment in *B. oleracea* genotypes. **Note:** A = the main founder rapid cycling line (DHSL150), B = *B. bourgaei* (C07007-S1), C = C10025-DH; G = C13013-DH; I = C13001-DH; J = *B. oleracea*-S1(C07060); L = C10128-DH; P = *B. oleracea*-S1 (C07079A); and Q = C10121-DH. Data represent mean (n = 3) and error bars show the standard error of the mean (SEM). Significant different by comparing the mean of untreated control plant vs treated determine using **paired one-tailed** Students t-test as indicated by an asterisk (* = p < 0.05; ** = 0.001). Different colours represent; black = control DHSL150; light brown = treated DHSL150, dark red = treated wild S1 lines, blue = untreated control wild S1s, yellow = treated DH lines and navy blue = untreated control DH lines respectively.

The plants were allowed to grow for two additional weeks post the two-week treatment and harvested at week-ten of growth (10wk) after regular watering at three-days interval, (i.e., twice in a week). The results (**Figure 4.7**) revealed that the *B. oleracea* genotypes were better able to reduce the level of Na⁺ ion as compared with their first treatment at week-six. This indicates that the *B. oleracea* genotypes activate mechanisms to forestall the effect of excess Na⁺ ion on the essential functions such as photosynthesis.

The DH line C10121-DH showed a significant increase in K^+ level ($p < 0.05$). This further demonstrates the presence of unique mechanisms that enable it to take Na^+ , retained more K^+ as well as Ca^{2+} levels after salt treatment. Moreover, this would also explain the gradual reduction of Na^+ and improving K^+ level two weeks post-treatment in all the genotypes (Figure 4.7).

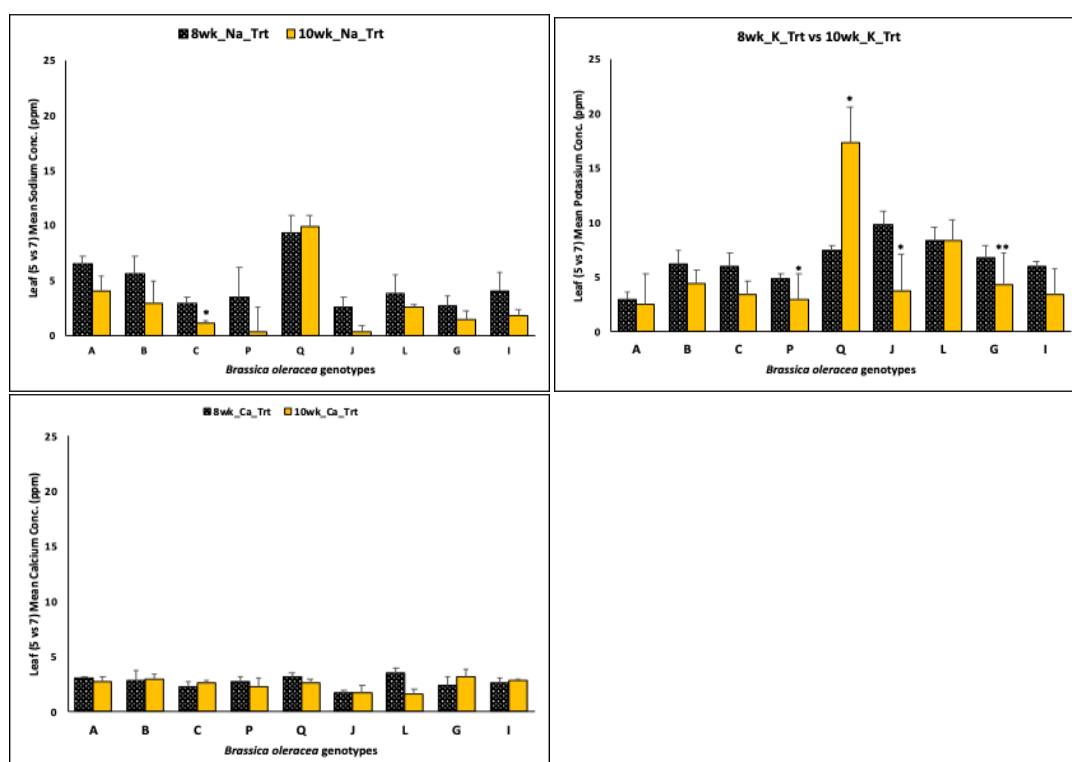


Figure 4.7: A comparison of Na^+ , K^+ and Ca^{2+} level between 2 weeks post initial salt treatment and 2 weeks post second salt treatment in *B. oleracea* genotypes. **Legend:** black bars = 2 weeks post initial salt treatment and navy blue = 2 weeks post second salt treatment on the same genotypes. Data represents mean ($n = 3$) and error bars show the standard error of the mean (SEM). Significant different by comparing the mean of untreated control plant vs treated determine using **paired one-tailed** Students t-test as indicated by an asterisk (* = $p < 0.05$; ** = 0.001). **Note:** A = the main founder rapid cycling line (DHLS150), B = *B. bourgaei*-S1 (C07007-S1), C = C10025-DH; G = C13013-DH; I = C13001-DH; J = *B. oleracea*-S1(C07060); L = C10128-DH; P = *B. oleracea*-S1 (C07079A); and Q = C10121-DH.

To correctly follow and understand the physiological variation in *B. oleracea* genotypes exposed to salt treatment at different stages and time, **Figure 4.8** was put together using all the time-points. It shows that the wildtype S1, cultivated founder DHLS150, and DH *B. oleracea* genotypes displayed similar physiological mechanism in response to salt stress. However, such mechanism becomes compromised in the presence of excess Na^+ as a result of salt treatment. The treated plants have shown to have a significant increase in Na^+ twenty-four hours post-treatment. Both parental lines; i.e., the wildtype S1, and founder DHLS150 and including some of the doubled haploid lines have shown to retained Na^+ , as a result, K^+ observed to be affected as compared to control untreated plants.

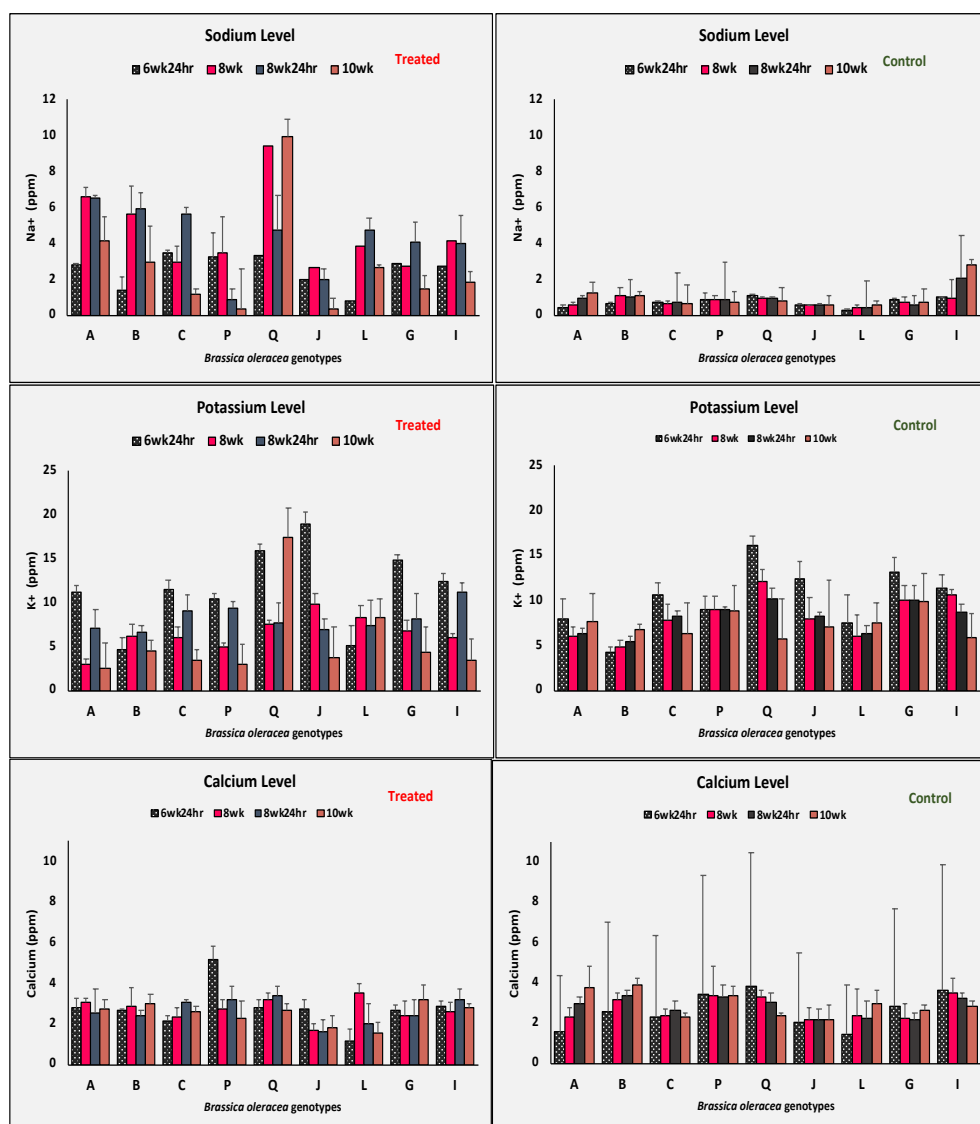


Figure 4.8: A summary of physiological variation observed in *B. oleracea* genotypes in response to salt shock stress at using four time-points data. Legend: black bars with dots = 24 hr post-treatment, red bars = 2 weeks post-treatment, dark grey = 24 hr post second treatment and brown = 2 weeks post second treatment. bars are means (n = 3) and error bars are standard error of the mean (SEM).

The Ca²⁺ level remained relatively unchanged despite salt treatment. This could suggest that Na⁺-Ca²⁺ interaction does not affect Ca²⁺ level in *B. oleracea* genotypes under salt stress. C10121-DH, was shown to have higher both Na⁺ and K⁺ ions level also maintaining Ca²⁺ level. The possible mechanism employed by this DH line has shown to differ from the parent lines where K⁺ level dropped in detriment to Na⁺ two-weeks post-treatment.

4.2.4 Na⁺/K⁺ Ratio (second salt screening)

Other important indicators for salt stress tolerance evaluated are K⁺/Na⁺ and Ca²⁺/Na⁺ ratios. Salt stress generally has shown to affect K⁺/Na⁺ ratio due to an increased influx of Na⁺, however, in *B. oleracea* genotypes K⁺/Na⁺ ratio remain high twenty-four hours after salt treatment as presented in **Table 4.2**. The highest K⁺/Na⁺ values observed was from parental wildtype S1 line *B. oleracea*-S1(C07060-S1) with 9.55 followed by a doubled haploid line C10128-DH genotype. This could suggest that these *B. oleracea* lines were able to cushion the effects associated with excess salt by high K⁺ within the plant cell systems.

Two-weeks post-treatment, due to the effects of salt stress on ion homeostasis K⁺/Na⁺ ratio has shown to be affected by increasing Na⁺ level against K⁺ thereby reducing the K⁺/Na⁺ ratio (**Table 4.2**) indicating susceptibility to salt stress.

Table 4.2: Comparison between K⁺/Na⁺, and Ca²⁺/Na⁺ Ratio 24 hrs and two-weeks post-treatments using 250 mM NaCl in *B. oleracea* genotypes

Geno ID	Species	24hr pt_K:Na	24hr pt_Ca:Na	2wk pt_K:Na	2wk pt_Ca:Na	Response
C10001	<i>B. ole(DHSL150)</i>	4.019	1.000	0.453	0.472	Susceptible
C13013-DH		5.241	0.934	2.471	0.879	Susceptible
C10025-DH		3.315	0.625	2.064	0.797	Susceptible
C10121-DH		4.782	0.844	0.798	0.341	Susceptible
C13001-DH		4.547	1.055	1.476	0.644	Susceptible
C07079A	<i>B. oleracea-S1</i>	3.191	1.604	1.408	0.794	Susceptible
C07060	<i>B. oleracea-S1</i>	9.554	1.389	3.755	0.644	Susceptible
C07007	<i>B. bourgaei-S1</i>	3.444	1.956	1.103	0.513	Susceptible
C10128-DH		6.608	1.525	2.169	0.923	Susceptible

Note: 24 hr pt = ratios 24 hr of post salt treatment and 2 wk pt = ratios 2 weeks post-treatment.

Following the second salt treatment at week-eight of growth, the *B. oleracea* genotypes were shown to improve their salt resilience by improving their K⁺/Na⁺ ratio (**Table 4.3**), when compared to earlier 8wk24hr K⁺/Na⁺ and 10wk_K: Na ratios presented (**Table 4.3**). The K⁺/Na⁺ ratio improved in most of the *B. oleracea* genotypes two-weeks post-treatment. An indication which could suggest activation of defence mechanisms to mitigate excess Na⁺ effects. Such improvements are observed in the wildtype S1 lines; *B. oleracea*-S1 (C07060)

from 3.450 to 12.061, and *B. bourgaei*-S1 (C07007-S1) from 1.122 to 1.516 respectively, while in the founder line DHLS150 reduced from 1.083 to 0.604 and *B. oleracea*-S1(C07079A-S1) 11.119 to 8.728. Also, an improvement in K^+/Na^+ ratio have been observed among the doubled haploid lines; C10128-DH from 1.57 – 3.146, C10025-DH from 1.595 – 3.014, C13013-DH from 1.988 – 2.895, C10121-DH from 1.636 – 1.759, and finally C13001-DH, which shows a decrease from 2.819 – 1.871.

Table 4.3: A comparison between K^+/Na^+ and Ca^{2+}/Na^+ Ratios after second salt treatment.

Geno ID	Species	8wk24hr pt_K:Na	8wk24hr pt_Ca:Na	10wk_K:Na	10wk_Ca:Na	Response
C10001	<i>B. ole(DHSL150)</i>	1.083	0.393	0.604	0.659	Susceptible
C13013-DH		1.988	0.596	2.895	2.187	Tolerant
C10025-DH		1.595	0.549	3.014	2.294	Tolerant
C10121-DH		1.636	0.722	1.759	0.269	Mildly Tolerant
C13001-DH		2.819	0.811	1.871	1.531	Mildly Susceptible
C07079A	<i>B. oleracea-S1</i>	11.119	3.853	8.728	6.676	Tolerant
C07060	<i>B. oleracea-S1</i>	3.450	0.802	12.061	5.784	Tolerant
C07007	<i>B. bourgaei-S1</i>	1.122	0.410	1.516	1.009	Mildly Tolerant
C10128-DH		1.572	0.426	3.146	0.590	Tolerant

• **Note:** 8wk24hr = 24hr post-treatment at 8 weeks and 10wk = two-weeks post-treatment.

The Ca^{2+}/Na^+ ratio remains unchanged. Generally, *B. oleracea* genotypes used have shown the ability to retain Ca^{2+} level despite excess salt. This could suggest the key role plays by calcium to ameliorate the effects of salt thereby improving salt tolerance in *B. oleracea* lines and also coupled to its function as a secondary messenger for growth and hormones activities.

4.2.5 Comparison between Morphological and Physiological Traits

Following the analyses of both morphological and physiological traits, significant variation between *B. oleracea* genotypes in response to salt stress was observed. An effort has been made to compare the two measurements with the view to unravel possible relationships

based on the observed variation. Therefore, a Pearson correlation analysis was conducted between the two traits in all the individual *B. oleracea* lines. The strength of the correlation (R^2) will be used to compare with between and the observed phenotype and to speculate possible mechanistic relations. Parents line and their derived DH lines will also be compared so as to derive any shared physiological mechanism between the parents and DH lines respectively.

From the analysis, the cultivated rapid cycling DHLS150 line was shown to be weakly correlated in plant height, leaf fresh weight and strong in dry weight in relation to Na^+ , K^+ ($R^2 = 0.273, 0.639 \text{ \& } 0.937$), and negatively correlated with respect to leaf area (Table 4.4). We observed an increase in both leaf fresh and dry weights in DHSL150. In contrast, leaf area correlates negatively ($R^2 = -0.0761$) and that could be as a result of high salt (Na^+) absorbed as it affects cell expansion and stomatal closure. In one of the DH line; C13013-DH derived from the DHSL150, has shown positive correlation in both plant height and fresh weight with respect to Na^+ level ($R^2 = 0.318 \text{ \& } 0.784$) and had also correlated negatively to K^+ level. This could explain the differences observed between the parent line DHSL150 and the DH line.

Table 4.4: A Pearson correlation between morphological and physiological traits in the cultivated DHSL150 and DH line C13013-DH

DHSL150	24hr_Na	24hr_K	24hr_Ca	2wk_Na	2wk_K	2wk_Ca	8wk24hr_Na	8wk24hr_K	8wk24hr_Ca	10wk_Na	10wk_K	10wk_Ca	Plant_height	Leaf_fresh_weight	Leaf_dry_weight	Leaf area
24hr_Na	1.0000	0.5711	0.8247	0.9730	-0.8728	-0.1540	-0.9921	-0.7112	0.5419	0.6852	0.8730	0.3812	0.2736	0.6398	0.9377	-0.0761
24hr_K		1.0000	0.0066	0.3662	0.9380	0.2760	-0.6839	-0.9583	-0.7028	-0.3368	0.3354	0.7399	0.9458	-0.5127	-0.8234	0.9065
24hr_Ca			1.0000	0.9329	0.4864	0.1420	-0.9737	0.4117	0.9345	0.9761	-0.1318	0.9386	-0.3184	0.7697	-0.9612	0.9881
2wk_Na				1.0000	-0.9941	0.1538	-0.6147	-0.9603	-0.9507	-0.9773	-0.5077	-0.5273	0.0442	-0.1834	-0.9804	0.9103
2wk_K					1.0000	0.9949	-0.4656	0.8956	0.6059	0.3603	0.6022	0.5530	-0.9195	0.7123	0.9527	-0.9981
2wk_Ca						1.0000	0.2193	-0.3533	-0.7595	0.6938	0.9835	-0.0349	-0.9925	-0.5383	-0.9112	0.7968
8wk24hr_Na							1.0000	0.8487	0.3274	-0.9999	-0.1307	0.6249	-0.9230	-0.2565	-0.9997	0.9221
8wk24hr_K								1.0000	0.6105	-0.3793	-0.6293	-0.2819	-0.9195	0.7123	0.9527	-0.9981
8wk24hr_Ca									1.0000	0.0631	-0.2211	0.9245	-0.9925	-0.5383	-0.9112	0.7968
10wk_Na										1.0000	-0.2467	-0.8792	-0.9093	-0.8508	-0.8486	0.9540
10wk_K											1.0000	0.1062	0.6275	0.7684	-0.6495	
10wk_Ca												1.0000	-0.7076	-0.8514	-1.0000	0.8322
Plant_height													1.0000	-0.2751	-0.1887	0.9874
Leaf_fresh_weight														1.0000	-0.3949	0.9600
Leaf_dry_weight															1.0000	0.9878
Leaf area																1.0000

C13013-DH	24hr_Na	24hr_K	24hr_Ca	2wk_Na	2wk_K	2wk_Ca	8wk24hr_Na	8wk24hr_K	8wk24hr_Ca	10wk_Na	10wk_K	10wk_Ca	Plant_height	Leaf_fresh_weight	Leaf_dry_weight	Leaf area
24hr_Na	1.0000	-0.0240	-0.0951	-0.2352	0.9537	0.8389	0.9794	0.9472	-0.6678	-0.5166	0.9717	-0.9120	0.3188	0.7844	-0.3804	0.1977
24hr_K		1.0000	-0.5052	-0.5997	-0.9494	0.1723	0.1891	-0.2556	-0.1978	0.1393	-0.3382	-0.8690	-0.8130	0.0797	-1.0000	0.9914
24hr_Ca			1.0000	-0.3876	0.2087	0.1542	0.9796	0.6648	-0.9299	-0.5916	0.9942	-0.6173	0.9132	0.0614	-0.3794	0.6564
2wk_Na				1.0000	0.0680	-0.5975	-0.9198	-0.5509	0.9955	0.8124	-0.9997	-0.8806	0.8463	-0.5201	0.5562	-0.7577
2wk_K					1.0000	1.0000	0.7387	0.2998	0.3822	0.9067	0.9490	-0.8522	0.5891	0.9956	-0.1856	-0.1272
2wk_Ca						1.0000	-0.6228	0.7265	0.5827	-0.9380	-0.9040	0.9990	-0.0840	0.6288	-0.8363	
8wk24hr_Na							1.0000	-0.8174	-0.9065	0.2641	0.9953	-0.9951	-0.1962	-0.8389	0.9015	
8wk24hr_K								1.0000	-0.9213	-0.9245	0.8480	0.6307	-0.2884	-0.9989	-0.8904	
8wk24hr_Ca									1.0000	0.9942	-0.6173	-0.9738	-0.6290	-0.5964	-0.1217	
10wk_Na										-0.9997	-0.5393	0.9351	0.6585	-0.9946	0.7413	
10wk_K											1.0000	0.5601	-0.8028	0.5540	-0.1192	-0.9151
10wk_Ca												1.0000	-0.8028	0.5540	-0.1192	-0.9151
Plant_height													1.0000	-0.1887	0.9874	-0.9452
Leaf_fresh_weight														1.0000	-1.0000	0.9769
Leaf_dry_weight															1.0000	-0.7778
Leaf area																1.0000

Key: Morphological Traits: PH = Plant Height, LFW = Leaf Fresh Weight, LDW = Leaf Dry weight, LA = Leaf Area. Physiological Traits: 24hr_Na = Na⁺ 24hr post 1st salt treatment, 24hr_K = K⁺ 24hr post 1st salt treatment, 24hr_Ca = Ca²⁺ 24hr post 1st salt treatment, 8wk_Na = Na⁺ 2 weeks post 1st salt treatment, 8wk_K = K⁺ 2 weeks post 1st salt treatment, 8wk_Ca = Ca²⁺ 2 weeks post 1st salt treatment, 8wk24hr_Na = Na⁺ 24hr post 2nd salt treatment, 8wk24hr_K = K⁺ 24hr post 2nd salt treatment, 8wk24hr_Ca = Ca²⁺ 24hr post 2nd salt treatment, 10wk_Na = Na⁺ 2 weeks post 2nd salt treatment, 10wk_K = K⁺ 2 weeks post 2nd salt treatment, 10wk_Ca = Ca²⁺ 2 weeks post 2nd salt treatment.

Similarly, the wild S1 line; *B. bourgaei*-S1 (C07007) has shown a positive correlation between plant height, leaf fresh and Na^+ , K^+ , and Ca^{2+} (Table 4.5) but correlates negatively in leaf dry weight and leaf area. This is in contrast to what was observed in the DHSL150, which shows a positive relation in Na^+ level and reduction in plant height. The wild S1 C07007 has shown an improved growth against its control line. This may further suggest clear differences in both physiology and morphological mechanisms employed by the parents' lines to handle excess Na^+ and at the same time improved growth. One of their derived DH line; C10025-DH that shows susceptibility in growth has shown a negative correlation between Na^+ and plant height and had reduced leaf dry weight and leaf area, both correlate positively with the level of Na^+ and K^+ . This corroborates our argument that the DH lines may show similar mechanisms in both morphological and physiology, however, differ to some certain extent due to possible allelic recombination and introgression.

Table 4.5: A Pearson correlation between morphological and physiological traits in the wild S1 *B. bourgaei*-S1 (C07007) and DH line C10025-DH

C07007-S1	24hr_Na	24hr_K	24hr_Ca	2wk_Na	2wk_K	2wk_Ca	8wk24hr_Na	8wk24hr_K	8wk24hr_Ca	10wk_Na	10wk_K	10wk_Ca	Plant_height	Leaf_fresh_weight	Leaf_dry_weight	Leafarea
24hr_Na	1.0000	0.9995	0.9927	-0.9879	-0.3412	0.5402	0.0377	-0.7827	-0.8174	-0.9065	0.2641	0.9953	0.5026	0.9703	-0.5435	-0.3744
24hr_K		1.0000	0.9886	-0.9828	-0.3694	0.8999	-0.9999	-0.7608	-0.3202	0.3576	0.4571	0.3482	0.5285	0.9772	-0.9017	-0.4022
24hr_Ca			1.0000	-0.9994	-0.2251	-0.2366	0.7295	0.9375	0.1559	0.7534	0.8962	0.7977	0.3945	0.9341	0.2404	-0.2596
2wk_Na				1.0000	0.1915	-0.2840	0.0701	-0.7371	0.4378	0.0561	0.9846	0.9143	-0.3627	-0.9212	0.2802	0.2262
2wk_K					1.0000	-0.4874	0.8858	0.8834	0.0151	0.8431	0.8986	0.8509	-0.9841	-0.5583	-1.0000	0.9994
2wk_Ca						1.0000	0.2908	-0.5984	0.5220	-0.1863	-0.5111	0.9789	0.6346	0.9965	-0.8840	-0.5181
8wk24hr_Na							1.0000	-0.7371	0.4378	0.0561	0.9846	0.9143	-0.2964	-0.8917	0.9924	0.1574
8wk24hr_K								1.0000	0.0151	0.8431	0.8986	0.8509	0.8639	0.9632	0.3240	-0.7834
8wk24hr_Ca									1.0000	-0.6780	-0.9690	-0.9569	0.5167	-0.2543	0.7819	-0.6333
10wk_Na										1.0000	-0.0037	0.5838	0.9240	0.3693	0.9035	-0.9690
10wk_K											1.0000	0.9994	0.8429	0.2011	0.9997	-0.9109
10wk_Ca												1.0000	0.9999	0.6857	0.9207	-0.9918
Plant_height													1.0000	0.6967	-0.9923	-0.9898
Leaf_fresh_weight														1.0000	0.0270	-0.5874
Leaf_dry_weight															1.0000	-0.8249
Leafarea																1.0000
C10025-DH	24hr_Na	24hr_K	24hr_Ca	2wk_Na	2wk_K	2wk_Ca	8wk24hr_Na	8wk24hr_K	8wk24hr_Ca	10wk_Na	10wk_K	10wk_Ca	Plant_height	Leaf_fresh_weight	Leaf_dry_weight	Leafarea
24hr_Na	1.0000	-0.3469	-0.7430	-0.8869	-0.9747	0.2557	-0.5239	0.8424	0.4088	0.6021	0.9964	0.9989	-0.9741	-0.9976	0.1670	0.9301
24hr_K		1.0000	-0.3699	-0.5335	0.3336	0.8828	0.8847	0.9339	0.9974	0.8780	0.9391	0.0298	0.1258	0.2810	0.9964	-0.1884
24hr_Ca			1.0000	-0.8533	-0.9845	-0.9986	-0.7597	-0.5802	-0.2212	-0.2302	0.4831	-0.6040	0.8751	0.7877	-0.6037	-0.9887
2wk_Na				1.0000	-0.3045	0.6708	-0.9621	-0.3178	0.9838	0.9881	0.9653	0.3606	0.8085	0.8913	0.9882	0.5788
2wk_K					1.0000	0.8082	0.9549	0.9123	0.9587	-0.6681	-0.7278	0.9115	-0.7576	-0.6449	-0.7723	-0.3121
2wk_Ca						1.0000	-0.6965	-0.4054	0.6646	0.8526	0.4885	0.9296	-0.3622	-0.2103	-0.3871	0.0482
8wk24hr_Na							1.0000	0.7042	0.6198	-0.7165	-0.9182	-1.0000	0.6337	0.5035	0.6527	0.6717
8wk24hr_K								1.0000	0.9974	0.8780	0.9391	0.0298	0.9435	0.9840	0.9073	0.4534
8wk24hr_Ca									1.0000	0.0211	-0.7993	-0.8664	0.4538	0.9943	0.9979	0.7321
10wk_Na										1.0000	0.0775	-0.6478	0.9005	0.1273	0.1248	0.5454
10wk_K											1.0000	0.0298	-0.9345	-0.9790	-0.8367	-0.9745
10wk_Ca												1.0000	0.3279	0.1745	0.1882	0.5025
Plant_height													1.0000	0.9874	-0.9452	0.2512
Leaf_fresh_weight														1.0000	0.4714	-0.9945
Leaf_dry_weight															1.0000	-0.0184
Leafarea																1.0000

Key: Morphological Traits: PH = Plant Height, LFW = Leaf Fresh Weight, LDW = Leaf Dry weight, LA = Leaf Area. Physiological Traits: 24hr_Na = Na⁺ 24hr post 1st salt treatment, 24hr_K = K⁺ 24hr post 1st salt treatment, 24hr_Ca = Ca²⁺ 24hr post 1st salt treatment, 8wk_Na = Na⁺ 2 weeks post 1st salt treatment, 8wk_K = K⁺ 2 weeks post 1st salt treatment, 8wk_Ca = Ca²⁺ 2 weeks post 1st salt treatment, 8wk24hr_Na = Na⁺ 24hr post 2nd salt treatment, 8wk24hr_K = K⁺ 24hr post 2nd salt treatment, 8wk24hr_Ca = Ca²⁺ 24hr post 2nd salt treatment, 10wk_Na⁺ = 2 weeks post 2nd salt treatment, 10wk_K = K⁺ 2 weeks post 2nd salt treatment, 10wk_Ca = Ca²⁺ 2 weeks post 2nd salt treatment.

The wild S1 *B. oleracea*-S1 (C07060) also showed a reduction in plant height and leaf area that correlated negatively with Na^+ , K^+ , and Ca^{2+} (Table 4.7). This differ compared to its DH line, C10128-DH which showed a positive correlation in both plant height and leaf dry weight. Both the parent line and DH line gave a negative correlation between leaf area which corroborates with morphological effects observed in the reduction of leaf area associated with excess salt.

Table 4.6: A Pearson correlation between morphological and physiological traits in the wild S1 *B. oleracea* (C07079A) and DH line C10121-DH

C07079A-S1	24hr_Na	24hr_K	24hr_Ca	2wk_Na	2wk_K	2wk_Ca	8wk24hr_Na	8wk24hr_K	8wk24hr_Ca	10wk_Na	10wk_K	10wk_Ca	Plant_height	Leaf_fresh_weight	Leaf_dry_weight	Leaf area
24hr_Na	1.0000	0.4955	-0.8802	-0.9957	-0.3045	0.6708	-0.9621	-0.3178	0.9838	0.9881	0.9653	0.3606	0.9952	0.3889	0.6862	-0.9993
24hr_K		1.0000	-0.0240	0.7640	0.9447	-0.8027	-0.2333	0.3696	0.3928	0.9307	0.9681	0.2819	0.4082	0.2073	-0.9937	0.9875
24hr_Ca			1.0000	-0.9455	-0.6561	-0.9504	-0.6965	-0.4054	0.6646	0.8526	0.4885	0.9296	-0.9224	-0.4728	-0.9019	-0.6986
2wk_Na				1.0000	0.7610	0.6818	-0.9881	0.7042	0.6198	-0.7165	-0.9182	-1.0000	0.4043	-0.9952	-0.9916	0.6598
2wk_K					1.0000	-0.8027	-0.2333	0.3696	0.3928	0.9307	0.9681	0.2819	-0.5751	-0.0572	0.9972	-0.2632
2wk_Ca						1.0000	-0.9986	-0.3301	-0.6960	-0.8706	-0.8714	-0.9998	0.9495	0.9842	0.4381	0.7838
8wk24hr_Na							1.0000	0.5513	0.9758	0.9999	-0.7932	0.9817	-0.9648	-0.4923	-0.9504	-0.3769
8wk24hr_K								1.0000	0.3928	0.9307	0.9681	0.2819	-0.7513	-0.2900	-0.9537	0.8630
8wk24hr_Ca									1.0000	-0.8706	-0.8714	-0.9998	-0.9020	-0.6201	-0.9986	0.7280
10wk_Na										1.0000	-0.7932	0.9817	0.9977	0.1554	0.9974	0.4363
10wk_K											1.0000	0.2819	-0.8327	-0.0358	0.9496	-0.5470
10wk_Ca												1.0000	0.2965	0.6221	0.9659	-0.7756
Plant_height													1.0000	0.4513	0.9263	-0.8745
Leaf_fresh_weight														1.0000	-1.0000	-1.0000
Leaf_dry_weight															1.0000	0.8571
Leaf area																1.0000
C10121-DH	24hr_Na	24hr_K	24hr_Ca	2wk_Na	2wk_K	2wk_Ca	8wk24hr_Na	8wk24hr_K	8wk24hr_Ca	10wk_Na	10wk_K	10wk_Ca	Plant_height	Leaf_fresh_weight	Leaf_dry_weight	Leaf area
24hr_Na	1.0000	0.9886	0.5928	0.6844	-0.9941	-0.7423	0.1065	-0.9406	-0.0549	-0.2283	-0.3114	0.0096	0.9317	0.9995	-0.9156	-0.9969
24hr_K		1.0000	0.9930	-0.0638	0.9156	0.9983	0.7895	0.9541	1.0000	0.9138	-0.7749	-0.3879	0.3724	-0.9436	0.3564	-0.6360
24hr_Ca			1.0000	-0.1815	-0.1565	0.2043	-0.4729	-0.8990	0.8204	0.9817	-0.7684	-0.4511	0.2599	0.0808	-0.8089	-0.8673
2wk_Na				1.0000	0.9880	0.9909	0.5826	0.6784	0.7750	0.8908	-0.2935	-0.9978	0.9024	-0.9971	0.4103	-0.0285
2wk_K					1.0000	0.9995	0.9975	0.1601	0.3706	0.0914	0.9493	-0.2454	0.9366	0.9930	0.8846	0.8139
2wk_Ca						1.0000	0.2618	0.6510	-0.1833	-0.9245	0.0694	0.7256	0.5022	-0.6403	0.9914	0.9236
8wk24hr_Na							1.0000	-0.1061	0.4893	0.7857	0.3777	0.5175	0.5245	0.9120	0.8880	0.9797
8wk24hr_K								1.0000	0.9745	0.9999	0.9976	0.9996	-0.9954	0.9892	0.6328	0.8790
8wk24hr_Ca									1.0000	0.9817	-0.7684	-0.4511	-0.9916	0.9906	0.5774	0.8644
10wk_Na										1.0000	0.9568	-0.7114	-0.9487	-0.6737	0.4683	0.7934
10wk_K											1.0000	0.9996	-0.9996	0.9986	0.3964	0.9842
10wk_Ca												1.0000	-1.0000	0.9837	0.2147	0.9784
Plant_height													1.0000	0.9429	0.3473	-0.6247
Leaf_fresh_weight														1.0000	-1.0000	1.0000
Leaf_dry_weight															1.0000	0.4670
Leaf area																1.0000

Key: Morphological Traits: PH = Plant Height, LFW = Leaf Fresh Weight, LDW = Leaf Dry weight, LA = Leaf Area. Physiological Traits: 24hr_Na = Na⁺ 24hr post 1st salt treatment, 24hr_K = K⁺ 24hr post 1st salt treatment, 24hr_Ca = Ca²⁺ 24hr post 1st salt treatment, 8wk_Na = Na⁺ 2 weeks post 1st salt treatment, 8wk_K = K⁺ 2 weeks post 1st salt treatment, 8wk_Ca = Ca²⁺ 2 weeks post 1st salt treatment, 8wk24hr_Na = Na⁺ 24hr post 2nd salt treatment, 8wk24hr_K = K⁺ 24hr post 2nd salt treatment, 8wk24hr_Ca = Ca²⁺ 24hr post 2nd salt treatment, 10wk_Na = Na⁺ 2 weeks post 2nd salt treatment, 10wk_K = K⁺ 2 weeks post 2nd salt treatment, 10wk_Ca = Ca²⁺ 2 weeks post 2nd salt treatment

Table 4.7: A Pearson correlation between morphological and physiological traits in the wild S1 *B. oleracea*-S1(C07060) and DH line C10128-DH

C07060-S1	24hr_Na	24hr_K	24hr_Ca	2wk_Na	2wk_K	2wk_Ca	8wk24hr_Na	8wk24hr_K	8wk24hr_Ca	10wk_Na	10wk_K	10wk_Ca	Plant_height	Leaf_fresh_weight	Leaf_dry_weight	Leafarea
24hr_Na	1.0000	0.3984	0.4542	0.6084	0.5743	0.8082	0.9549	0.9123	0.9587	-0.6681	-0.7278	0.9115	-0.4778	0.3354	-0.5674	-0.9185
24hr_K		1.0000	0.9981	0.9876	0.9757	0.8155	0.9579	0.9415	0.5354	-0.7168	-0.8819	-0.3155	-0.9961	-0.4053	-0.9775	-0.7286
24hr_Ca			1.0000	-0.9734	0.7610	0.6818	-0.9881	0.7042	0.6198	-0.7165	-0.9182	-1.0000	-0.9996	-0.5435	-1.0000	-0.7694
2wk_Na				1.0000	0.3183	0.8294	0.7208	0.2730	0.9977	0.9652	-0.6780	0.6740	-0.5987	0.1860	-0.6732	-0.9657
2wk_K					1.0000	0.8155	0.9579	0.9415	0.5354	-0.7168	-0.8819	-0.3155	0.5687	0.7031	-0.9978	-0.0612
2wk_Ca						1.0000	-0.0077	0.4298	0.9659	0.9832	0.9074	0.7713	-0.0122	0.4693	-0.9170	-0.6275
8wk24hr_Na							1.0000	0.2730	0.9977	0.9652	-0.6780	0.6740	-0.9998	-0.5957	-0.9890	-0.7737
8wk24hr_K								1.0000	0.5354	-0.7168	-0.8819	-0.3155	-0.2538	-0.5401	-0.9932	0.3982
8wk24hr_Ca									1.0000	0.9832	0.9074	0.7713	-0.9528	-0.1997	0.8211	-0.5615
10wk_Na										1.0000	-0.6780	0.6740	-0.8815	-0.5930	0.9966	-0.4013
10wk_K											1.0000	-0.3155	0.9447	0.1951	0.7901	0.9454
10wk_Ca												1.0000	-0.6093	-1.0000	0.9891	0.0111
Plant_height													1.0000	0.0999	0.9593	0.7862
Leaf_fresh_weight														1.0000	-1.0000	-0.1946
Leaf_dry_weight															1.0000	-0.8667
Leafarea																1.0000
C10128-DH	24hr_Na	24hr_K	24hr_Ca	2wk_Na	2wk_K	2wk_Ca	8wk24hr_Na	8wk24hr_K	8wk24hr_Ca	10wk_Na	10wk_K	10wk_Ca	Plant_height	Leaf_fresh_weight	Leaf_dry_weight	Leafarea
24hr_Na	1.0000	0.5150	0.5516	0.9920	0.7367	-0.7657	0.2618	0.6510	-0.1833	-0.9245	0.0694	0.7256	0.9635	-0.9231	0.9337	-0.9134
24hr_K		1.0000	0.9991	-0.9501	-0.8239	-0.9470	-0.4905	-0.8975	-0.2133	1.0000	0.5877	-0.5346	0.7256	-0.8051	0.1738	-0.8194
24hr_Ca			1.0000	0.6818	-0.9881	0.7042	0.6198	-0.7165	-0.9182	-1.0000	0.1418	0.0158	0.7547	-0.8299	0.2162	-0.8434
2wk_Na				1.0000	-0.9501	-0.8239	-0.9470	-0.4905	-0.8975	-0.2133	1.0000	0.5877	0.9221	-0.7327	0.9714	-0.8548
2wk_K					1.0000	-0.4197	0.9013	-0.8949	-0.9879	0.3354	0.5829	-1.0000	-0.7555	0.7015	-0.9970	0.6503
2wk_Ca						1.0000	-0.5276	-0.5263	-0.8270	-0.8722	-0.7457	-0.3936	0.9117	0.4884	0.4884	-0.9625
8wk24hr_Na							1.0000	-0.9901	-0.8998	0.6502	-0.9914	-0.9978	-0.1321	0.7895	0.4836	0.2772
8wk24hr_K								1.0000	0.1393	-0.3382	-0.8690	-0.9131	0.9286	0.9937	0.5256	-0.9733
8wk24hr_Ca									1.0000	-0.9997	-0.9894	-0.6419	-0.8597	0.9258	-0.3871	0.9258
10wk_Na										1.0000	0.9955	0.9617	0.5000	1.0000	-0.1134	-0.6226
10wk_K											1.0000	-0.5473	0.5000	-0.6031	-0.1134	-0.6226
10wk_Ca												1.0000	-0.5000	0.6031	0.1134	0.6226
Plant_height													1.0000	-0.9923	0.8038	-0.9890
Leaf_fresh_weight														1.0000	-0.7241	0.9997
Leaf_dry_weight															1.0000	-0.7070
Leafarea																1.0000

Key: Morphological Traits: PH = Plant Height, LFW = Leaf Fresh Weight, LDW = Leaf Dry weight, LA = Leaf Area. Physiological Traits: 24hr_Na = Na⁺ 24hr post 1st salt treatment, 24hr_K = K⁺ 24hr post 1st salt treatment, 24hr_Ca = Ca²⁺ 24hr post 1st salt treatment, 8wk_Na = Na⁺ 2 weeks post 1st salt treatment, 8wk_K = K⁺ 2 weeks post 1st salt treatment, 8wk_Ca = Ca²⁺ 2 weeks post 1st salt treatment, 8wk24hr_Na = Na⁺ 24hr post 2nd salt treatment, 8wk24hr_K = K⁺ 24hr post 2nd salt treatment, 8wk24hr_Ca = Ca²⁺ 24hr post 2nd salt treatment, 10wk_Na = Na⁺ 2 weeks post 2nd salt treatment, 10wk_K = K⁺ 2 weeks post 2nd salt treatment, 10wk_Ca = Ca²⁺ 2 weeks post 2nd salt treatment.

The morphological and physiological traits comparisons were combined to produce a comparative outlook with the view to establish a clear picture on how the data correlates with different traits as presented in **Figure 4.9**.

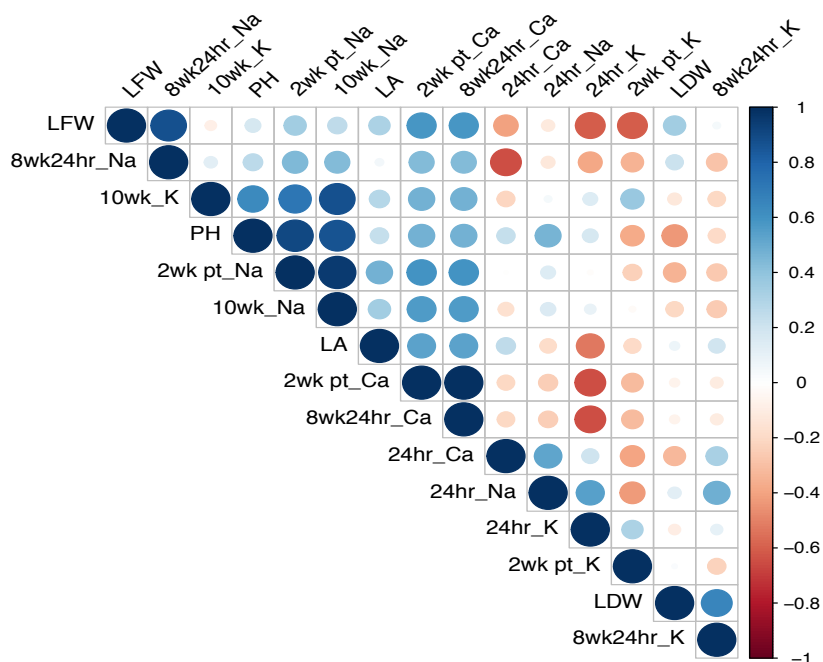


Figure 4.9: A general correlation outlook using the whole data representing relationship between morphological and physiological traits in *B. oleracea* genotypes following salt treatment at different physiological stages of plant growth. **Legend:** +1 to -1 represent perfect positive correlation to negative correlation. **Key:** Morphological Traits: PH = Plant Height, LFW = Leaf Fresh Weight, LDW = Leaf Dry weight, LA = Leaf Area. Physiological Traits: 24hr_Na = Na⁺ 24hr post 1st salt treatment, 24hr_K = K⁺ 24hr post 1st salt treatment, 24hr_Ca = Ca²⁺ 24hr post 1st salt treatment, 8wk_Na = Na⁺ 2 weeks post 1st salt treatment, 8wk_K = K⁺ 2 weeks post 1st salt treatment, 8wk_Ca = Ca²⁺ 2 weeks post 1st salt treatment, 8wk24hr_Na = Na⁺ 24hr post 2nd salt treatment, 8wk24hr_K = K⁺ 24hr post 2nd salt treatment, 8wk24hr_Ca = Ca²⁺ 24hr post 2nd salt treatment, 10wk_Na⁺ = 2 weeks post 2nd salt treatment, 10wk_K = K⁺ 2 weeks post 2nd salt treatment, 10wk_Ca = Ca²⁺ 2 weeks post 2nd salt treatment.

Principal component (PC) analysis was carried out on the data to determine the proportion of variances retained by the principal components. This was done by extracting the eigenvalues, which correspond to the amount of the variation explained by each principal component as presented in **Figure 4.10**.

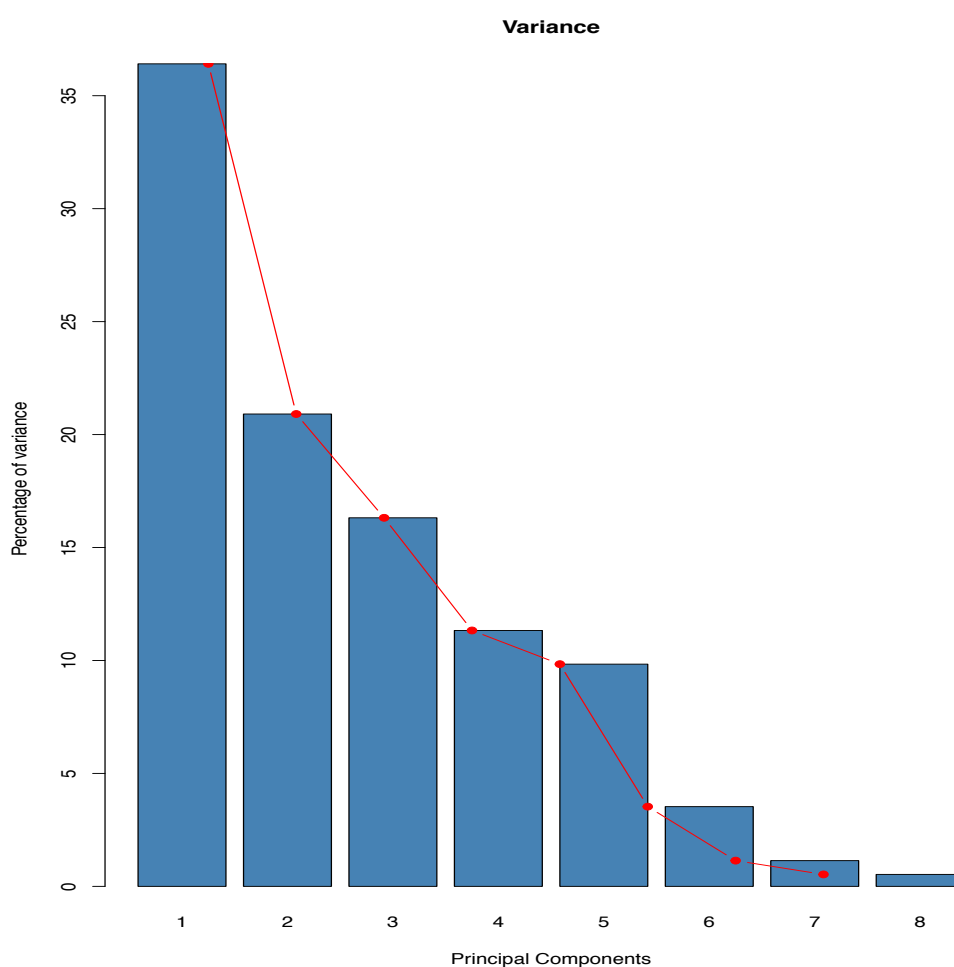


Figure 4.10: A plot of eigenvalues and variance associated with components generated from the data.

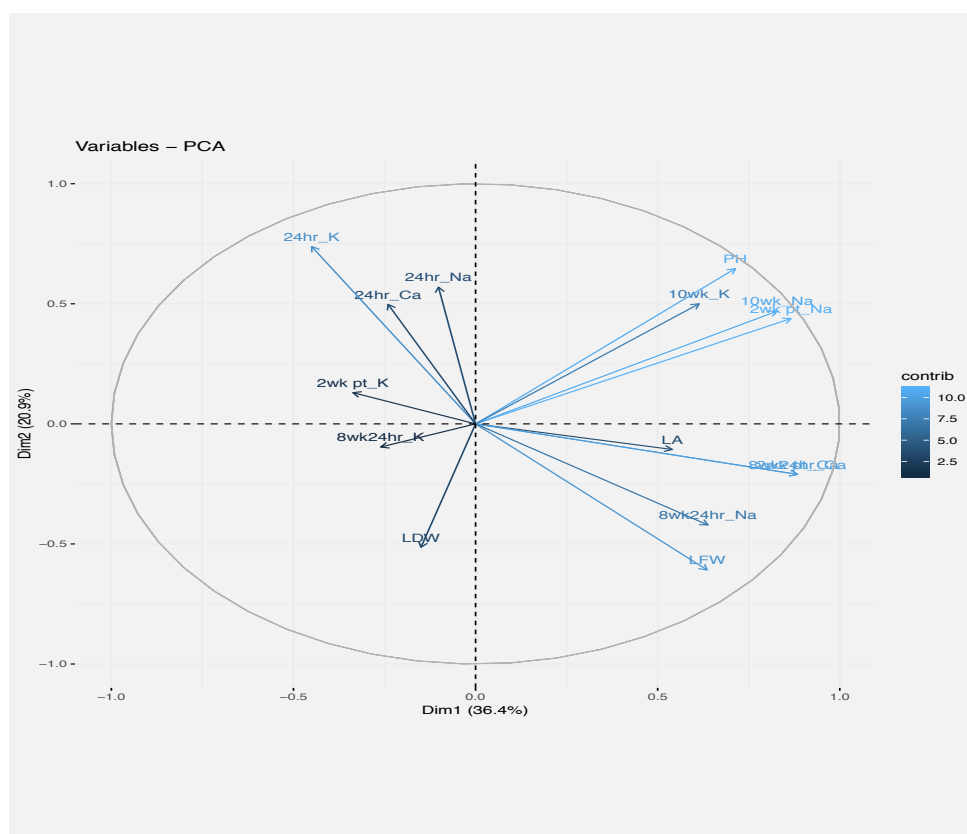


Figure 4.11: The strength of variables contribution in the formation of PC plot. **Legend:** light blue to dark blue indicate variable contribution from higher to lower. **Key:** Morphological Traits: PH = Plant Height, LFW = Leaf Fresh Weight, LDW = Leaf Dry weight, LA = Leaf Area. Physiological Traits: 24hr_Na = Na^+ 24hr post 1st salt treatment, 24hr_K = K^+ 24hr post 1st salt treatment, 24hr_Ca = Ca^{2+} 24hr post 1st salt treatment, 8wk_Na = Na^+ 2 weeks post 1st salt treatment, 8wk_K = K^+ 2 weeks post 1st salt treatment, 8wk_Ca = Ca^{2+} 2 weeks post 1st salt treatment, 8wk24hr_Na = Na^+ 24hr post 2nd salt treatment, 8wk24hr_K = K^+ 24hr post 2nd salt treatment, 8wk24hr_Ca = Ca^{2+} 24hr post 2nd salt treatment, 10wk_Na = Na^+ 2 weeks post 2nd salt treatment, 10wk_K = K^+ 2 weeks post 2nd salt treatment, 10wk_Ca = Ca^{2+} 2 weeks post 2nd salt treatment.

A biplot was also plotted to group the variables measured (the morphological and physiological traits) together with *B. oleracea* lines to enable draw relationships between the *B. oleracea* lines based on variables. **Figure 4.12.**

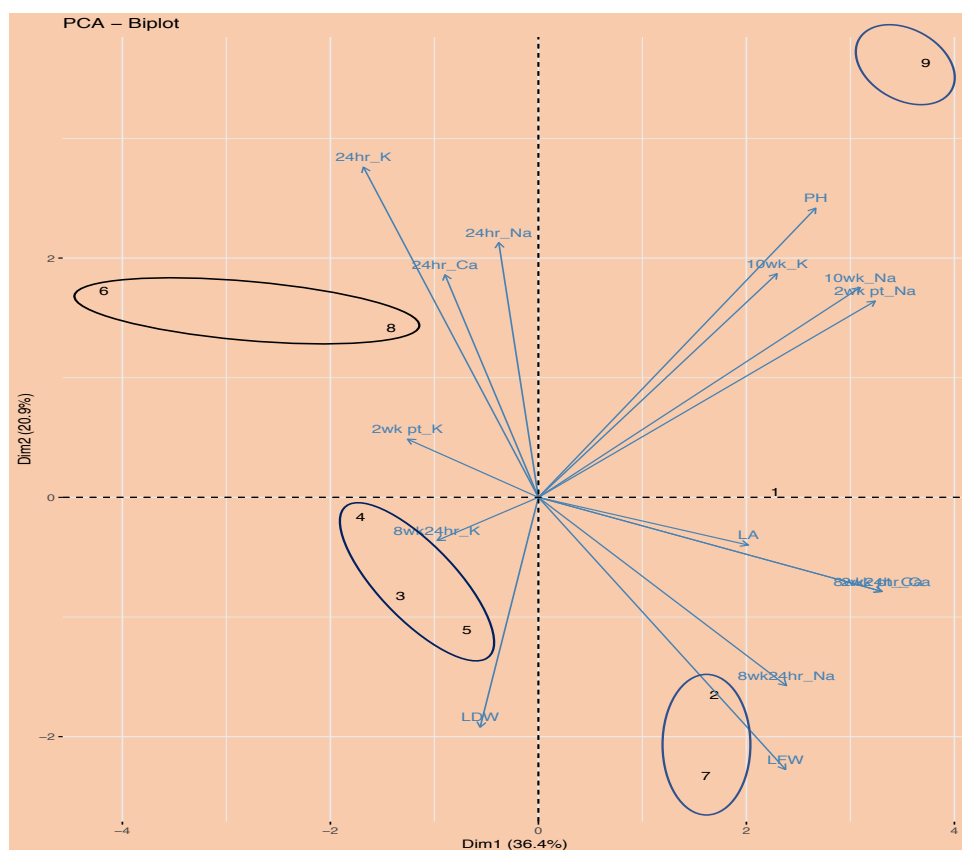


Figure 4.12: A biplot of variables and individual *B. oleracea* genotypes. Note: **1** = the founder rapid cycling line DHSL150, **2** = *B. bourgaei*-S1 (C07007), **3** = C10025-DH, **4** = C13013-DH, **5** = C13001-DH, **6** = *B. oleracea*-S1 (C07060), **7** = C10128-DH, **8** = *B. oleracea*-S1(C07079A) and **9** = C10121-DH. **Key:** Morphological Traits: PH = Plant Height, LFW = Leaf Fresh Weight, LDW = Leaf Dry weight, LA = Leaf Area. Physiological Traits: 24hr_Na = Na⁺ 24hr post 1st salt treatment, 24hr_K = K⁺ 24hr post 1st salt treatment, 24hr_Ca = Ca²⁺ 24hr post 1st salt treatment, 8wk_Na = Na⁺ 2 weeks post 1st salt treatment, 8wk_K = K⁺ 2 weeks post 1st salt treatment, 8wk_Ca = Ca²⁺ 2 weeks post 1st salt treatment, 8wk24hr_Na = Na⁺ 24hr post 2nd salt treatment, 8wk24hr_K = K⁺ 24hr post 2nd salt treatment, 8wk24hr_Ca = Ca²⁺ 24hr post 2nd salt treatment, 10wk_Na⁺ = 2 weeks post 2nd salt treatment, 10wk_K = K⁺ 2 weeks post 2nd salt treatment, 10wk_Ca = Ca²⁺ 2 weeks post 2nd salt treatment.

4.3 Discussion

Plants exposed to higher salt experience stress, both hyper-ionic and hyperosmotic through an accumulation of Na^+ and Cl^- ions, which causes membrane damage, nutrient imbalance, enzymatic inhibition, metabolic dysfunction, etc. The quick and basic response of plants exposed to higher salt has been K^+ efflux from the cells caused by excess Na^+ (Nedjimi and Daoud, 2009; Anschutz et al., 2014; Bose et al., 2014b). The huge Na^+ influx in the plant growth medium creates a plasma membrane depolarisation which further activates membrane-bound cation channels, the guard cell outward rectifying potassium channels (GORK), that stimulate Na^+ diffusion into the cell, and K^+ efflux and thereby increasing Na^+ content (Blumwald et al., 2000; Demidchik and Tester, 2002). Salt-induced stress creates disruption of the K^+/Na^+ ratio (Simaei et al., 2012) and interferes with K^+ homeostasis (Tunçtürk et al., 2008).

From our result, we observed that salt stress-induced causes an increase in Na^+ leaf content twenty-four hours post-treatment within the *B. oleracea* genotypes. Increase in Na^+ leaf content following salt stress has been reported in many studies by Essa (2002); Yasar et al. (2006); Kusvuran et al. (2007) and work on canola genotypes by Tunçtürk et al. (2011). The level of potassium was affected by two-weeks post-treatment. Reduction in potassium level due salt stress has been widely reported e.g., studies by Essa (2002); Ashraf and McNeilly (2004); Li et al. (2006); Yasar et al. (2006); Bandeh-Hagh et al. (2008); Rahman et al. (2016). Potassium plays a critical role in the neutralisation reactions of anion and regulates cell membrane polarisation, osmoregulation, likewise being an important factor in the activity of enzymes involved in many metabolic pathways (Very et al., 2014). The reduction of potassium level was attributed to the entry of the higher amount of Na^+ into plant roots cell by non-selective cation channels (NSCC) that cause K^+ efflux or leakage through guard

cell outward rectifying potassium channel (GORK) and stellar K^+ outward rectifying channel (SKOR) (Rahman et al., 2016). Other potassium transporters that might be implicated are membrane-bound protein channels actively involved in transportation of potassium like Shaker K^+ channel, High affinity potassium (HAK), potassium uptake (KUP), potassium transporter (KT) and high affinity potassium transporter (HKT) amongst others (Wang and Wu, 2013; Very et al., 2014; Shabala and Pottosin, 2014).

Higher Na^+ accumulation was observed two-weeks post-treatment leading to a reduction of K^+/Na^+ ratio, which might be as a result of disruption of ion homeostasis. The Na^+ influx and K^+ efflux have been associated with increased ROS production that could lead to the activation of NSCC (Maathius, 2006). A similar report showed that higher Na^+ lead to the disruption of ion homeostasis under salt stress conditions (Tunçtürk et al., 2008; Wu and Wang, 2012). From our result, Ca^{2+} level showed to be relatively unaffected in *Brassica oleracea* genotypes despite higher Na^+ in the growth medium. Moreover, it has been reported that exogenous calcium promotes membrane stability, thus ameliorating salt toxicity by decreasing Na^+ influx through NSCC and indirectly inhibiting K^+ efflux through GORK channel in plants (Cramer et al., 1985; Essa et al., 2003; Shabala et al., 2006; Nadjimi and Daoud, 2009; Shabala and Pottosin, 2014). More so, exogenous calcium has shown to cause a reduction in the uptake and transport of Na^+ and further preventing it from binding to the cell wall (Kurth et al., 1986; Rubio et al., 2003). Other functions of cellular and vacuolar calcium include blockage of the fast vacuole (FV) channel in a voltage-dependent and independent reaction preventing Na^+ from being leak back into vacuole and ultimately their transport into the cell (Tikhonova et al., 1997; Shabala, 2013).

Improved Ca^{2+} level observed in *B. oleracea* genotypes might be the cause of improved K^+ level observed after the plants were salt stress for the second time thus, leading to higher K^+/Na^+ ratio. Although an increase in Na^+ leaf content was observed, the *B. oleracea* genotypes were able to improve and retained their Ca^{2+} level unaffected. Ca^{2+} ion, in optimum concentrations, has been shown to play crucial roles in many physiological processes of plants and shown to increase plant resistance to abiotic stresses (Watkin et al., 2000). In addition, it has been suggested that Ca^{2+} participates in the regulatory mechanism of plants' adjustment to adverse conditions; high temperature, cold injury, drought stress and salt stress (Arora and Palta, 1989; Bowler and Fluhr, 2000; Mozafari et al., 2008; Joshi et al., 2012). It was reported also that higher extracellular $\text{Ca}^{2+}/\text{Na}^+$ ratio causes a reduction in Na^+ influx (Rengel, 1992).

4.4 Summary

Salt stress triggers a plant's physiological and morphological response for adaptation and growth. This chapter investigates the physiological variation in *B. oleracea* genotypes exposed to salt stress using 250 mM NaCl. The result demonstrates significant variation between the parent lines; DHSL150, the wild S1, and their derived DH lines. The level of different nutrients content; Na⁺, K⁺, and Ca²⁺ analysed have shown significant variation. Leaf Na⁺ content rises 24 hr post-treatment in all the *B. oleracea* lines, indicating physiological response related to an osmotic phase of salt stress whereby Na⁺ influxes causes cell-water imbalances. It also shows a delay in activation of Na⁺ exclusion channels that shown to recycle Na⁺ in and out, so as to reduce its accumulation in the plant cell further preventing it from reaching the important plant organs such as leaves. Furthermore, it is an indication that the *B. oleracea* lines share similar biochemical response in handling Na⁺ 24 hr post-treatment. Potassium (K⁺), an osmotically important element in plant cell plays a critical role in both structure and maintenance of cell turgor has shown to be relatively unaffected 24 hr post-treatment, so also the Ca²⁺ level, which remains unchanged. This chapter also reported the deteriorating effects of excess salt two-weeks post-treatment on K⁺ level, an indication of homeostasis effects of Na⁺ against K⁺, which is widely reported in many reviews that Na⁺ competes with K⁺ thereby displaces it to get into the cell especially through non-selective cation channels (SNCCs) and other low affinity K⁺ channels such as K⁺ outward-rectifying (KOR) channels and inward-rectifying (KIR) channels as a result of membrane depolarisation by excess Na⁺, which led to movement of Na⁺ and reduction of K⁺. These processes could be the cause of K⁺ reduction and increase Na⁺ observed in the *B. oleracea* lines. Although significant variation observed between genotypes, a statistical significant reduction was observed in the cultivated rapid cycling DHSL150 and a DH line (C13013) as against their untreated control lines while in wild S1 lines, and four other DH

lines have shown non-significant reduction as compared to their control lines. This could also be one of the reasons of high Na^+/K^+ ratio observed in these lines and high cellular Na^+/K^+ ratio is attributed salt resilience in many plants especially the salt tolerant ones. Different correlation analysis have been conducted to establish a relationship between the observed morphological variation and physiological response, different traits have shown both strong positive and negative correlation based on individual lines and this was further corroborated when the data was put together to creates to general outlook which confirms the individual correlations that the morphological variation observed is connected to physiological response. Principal component analysis conducted further group the lines based on the quality of variable contribution in PC generation and how it relates to individual lines where some DH lines were put together with the wild S1 parent in one quadrant of PC biplot. Signifies good morphological and physiological relationship in response to salt stress in these *B. oleracea* lines and both wild S1 and some DH lines have shown better performance to salt stress than the cultivated rapid cycling DHSL150. However, more work is recommended to enable their further classification based on salt resilience.

CHAPTER FIVE

Relative Gene Expression of Ion Membrane Transporters in *B. oleracea* genotypes in Response to Salt Stress

5.0 Introduction

As described in Chapter One, salt tolerance within plants involves reduction of excess Na^+ and retention of K^+ ions in the cytosol. This has been attributed to be regulated by the membrane ion transporters. These are involved in either removing the added Na^+ from cells through a membrane-bound Na^+/H^+ antiporter, and or via sequestration of excess Na^+ in vacuoles through the NHX or Na^+/H^+ antiporter in the vacuole membrane (Blumwald and Pool, 1985; Zhu, 2003; Bassil et al., 2012). These membrane transporters are also involved in K^+ sequestration within the vacuole. Both types are ubiquitous membrane proteins that catalyse the electroneutral exchange of Na^+ and or H^+ across membrane, thereby playing essential roles in cellular Na^+/K^+ and pH homeostasis (Rodriguez-Rosales et al., 2008; Leidi et al., 2010; Barragan et al., 2012). The calcium-permeable nonselective cation channels (NSCC) contribute both directly and indirectly to the Na^+ entry into the cell. The influx of Na^+ through these channels stimulates depolarisation of the plasma membrane, activating K^+ outward-rectifying channels (KOR) and thus reducing net passive K^+ uptake through the inward-rectifying K^+ channels (KIR) (Demidchik et al., 2003; Shabala and Cuin, 2008; Demidchik, 2014). Accumulation of cGMP suppresses influx of Na^+ by deactivation process via NSCC and allow the apoplastic Ca^{2+} into the cell cytoplasm through CNGC. Enhanced cytoplasmic Ca^{2+} typically indicates stresses (including; salt stress), and consequent triggers cascade reactions via activation of cytosolic calcium induces calmodulin (CaM)-dependent kinases, which further leads to activation of other plasma membrane H^+ -ATPases (Bose et al., 2014a). Variations in transcript levels of genes related to ion-transportation, photosynthesis, respiration, and other critical metabolic roles have been reported under saline conditions (Ozgur et al., 2013; Bose et al., 2014a,b). Thus, restoring membrane voltage and inhibiting depolarisation-activated NSCC and process that lead to the reduction

of Na^+ influx into the cell and efflux of K^+ , improve plant tolerance to salt stress have been considered to be important response mechanisms.

Investigation of these transporters with the aim of developing salt-tolerant plants has been widely reported. For example, High-Affinity Potassium Transporters (HKTs), which perform functions of Na^+/K^+ symporter and Na^+ uniporter present in the plasma membrane of different plant crops including wheat, rice, and *Arabidopsis* (Waters et al., 2013). These transporters have been shown to play a critical role in sodium recirculation in *Arabidopsis* from the shoots to roots by loading Na^+ from the shoot into the phloem and unloading it back into the roots (Berthomieu et al., 2003). *HKT1;4* isolated from a salt-tolerant durum wheat cultivar gene expressed in *Xenopus* oocytes has also been shown to exhibit high Na^+ selectivity (Amar et al., 2014). Studies by Sunarpi et al., (2005) indicated the localisation of HKTs to the plasma membrane of xylem parenchyma cells and linked it to salinity tolerance in *Arabidopsis*. It has also been shown that overexpression of HKT in *Arabidopsis HKT1;1* in the root stele causes reduction by 37-64% in resilient phenotype. In addition Salt overly sensitive transporters (see Chapter 1, Section 1.2.7.4 & **Figure 1.5**) such as (*SOS1*) have been shown to be involved in the transportation of Na^+ as indicated by studies carried out on mutant yeast that lacks Na^+ -ATPases and Na^+/H^+ antiporter activity (Shi et al., 2002a). Further studies indicated that *AtSOS1* transporters work as anti-porters in long-distance of Na^+ transport in *Arabidopsis* plants (Shi et al., 2002a,b). Overexpression of the *SOS1* gene isolated from a halophyte *Salicornia brachiata* has shown to improved salt tolerance in tobacco (Yadav et al., 2012).

An initial RNA-Seq study on RNA collected from four *B. oleracea* lines from my experiment; i.e., DHSL150, DH lines; C13013-DH, C10128-DH, and C13001-DH (see Chapters 1) provided a list of genes thought to vary in response to salt stress. The aim of

this chapter was to establish the variation in relative gene expression of a number of salt stress specific ion membrane transporters in *B. oleracea* genotypes identified from this list.

The chapter is set to achieve this by measuring the relative gene expression on the individual transcripts using sequence-specific primers designed to transcribe a short segment of transcript sequence and amplify using qPCR and normalised against specific housekeeping genes.

5.2 Results

5.2.1 Differentially Expressed Genes (DEGs)

During the analysis of RNA-Seq data, quality reads obtained from two of the doubled haploid lines; C13013-DH and C13001-DH that were mapped to the genome of To1000 *B. oleracea* and passed the DESeq2 statistics pipeline showed that ion membrane transporters were among the differential expressed genes 24 hr post-treatment. **Figure 5.1** shows expression of six plasma membrane-bound ion transporters within C13013-DH. One of these was the Na^+/H^+ ion exchanger isoform 1 (*NHX1*) (ID: Bo9g010200), which is involved in Na^+ ion compartmentalisation and exclusion from the root cells. The *NHX1* genes have shown higher relative expression in salt-treated line as compared to the untreated control. Another important electrochemical gradient proton generator that accomplishes the operation of tonoplast-bound NHX proteins; the vacuolar H^+ -ATPase (ID: Bo3g048430) was showed to have lower expression in the treated line compared to the untreated control. This contrasts to the expression of two calcium-transporting ATPase (*CaTATPase*) (ID: Bo8g091730 & Bo6g072820) located in chromosome 3 & 4 (Chr.3 and Chr.4) which exhibited higher expression as compared to the control plants (**Figure 4.4**). Our result further showed differential expression of vacuolar cation/proton exchanger (*V-CAX*) (ID: Bo4g145580) and Voltage-gated chloride channel (*V-CLC*) (ID: Bo4g145580) located in a chromosome, (Chr.3 and Chr.2) to be higher compared to the control lines. The activities of all these transporters have been associated with a plant's ability to reduce the effects of excess Na^+ and chloride ions within the cell cytoplasm (Natasha and Stephen, 2010).

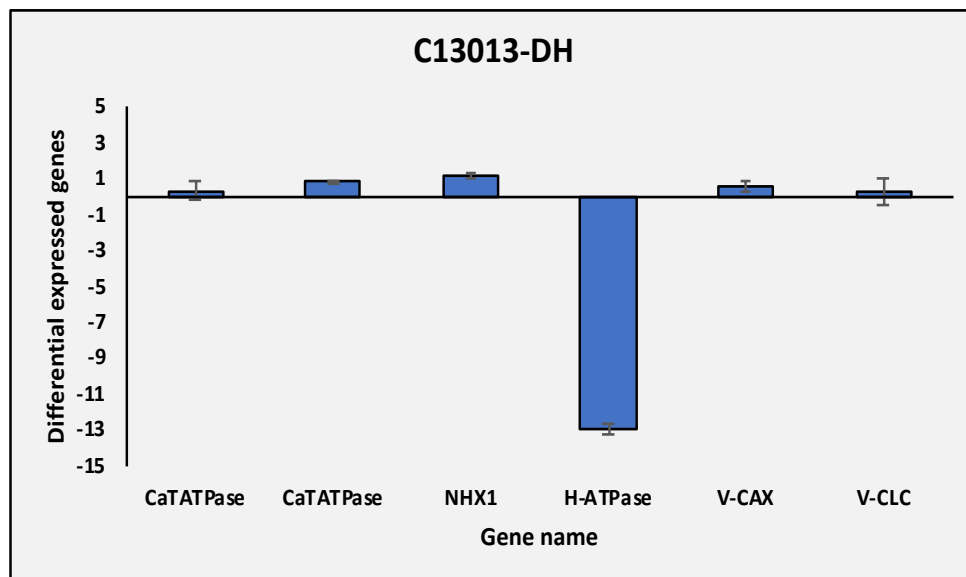


Figure 5.1: The ratio of differentially expressed genes related to Na^+/H^+ antiporter (*NHX1*), and Ca^{2+} homeostasis (CaTATPase), proton gradient (H-ATPase), vacuolar cation exchanger (*V-CAX*), and voltage-gated chloride channel (*V-CLC*) under salt stress in salt-treated *B. oleracea* DH genotype as against the salt untreated control. The values represent the ratio between control and treated ($n = 3$). Error bars are standard error of the mean (SEM).

The **Figure 5.1** shows the ratio of differential gene expression of these six ion membrane-bound transporters selected based on differential expression in treated plants as against the salt untreated control plants. The Na^+/H^+ antiporter (*NHX1*) showed 1.18-fold higher while the two isoforms of Ca^{2+} -ATPases exhibited respectively lower expression of 0.30 and 0.80 fold reduction as compared to untreated control (**Figure 5.1**). The H^+ -ATPase antiporters that are involved in cation exchange and regulation of electrochemical gradient were found to be suppressed by -12.93-fold in treated plants compared to control. The vacuolar cation exchanger (*V-CAX*) and voltage-gated chloride channel (*V-CLC*) were shown be expressed in treated by 0.51 and 0.24 folds less as compared to control untreated line.

Figure 5.2 shows a membrane ion transporters identified as being differential expressed in the C13001-DH genotype. This line varies in both morphological and physiological traits from the C13013-DH genotype (Chapters 3 and 4). The membrane channels that showed

differential expression in C13001-DH genotype are mainly tonoplast-bound protons generators that drive the electrochemical gradient and specifically vacuolar-type proton ATPases. The result showed up-regulation of these vacuolar ATPases in particular of different sub-units. Two G subunits, d2 and ATPase 116 kDa subunit-a, that perform similar role in the maintenance of pH by creating avenues for proton-driven accompanied by other critical transporters (Natasha and Stephen 2010). The expression was significant higher in all the following ion transporters: H⁺-ATPase (Bo9g027480), V-type proton subunit-G (Bo3g055180) and V-type subunit-a (Bo4g070380), which are showed to be located in chromosomes (Chr. 8, & Chr. 9) respectively. Two were shown to have lower expression compared to control, the V-type proton ATPase subunit-a (Bo7g091490) and G-subunit (Bo7g109030) both located on chromosome (Chr. 1).

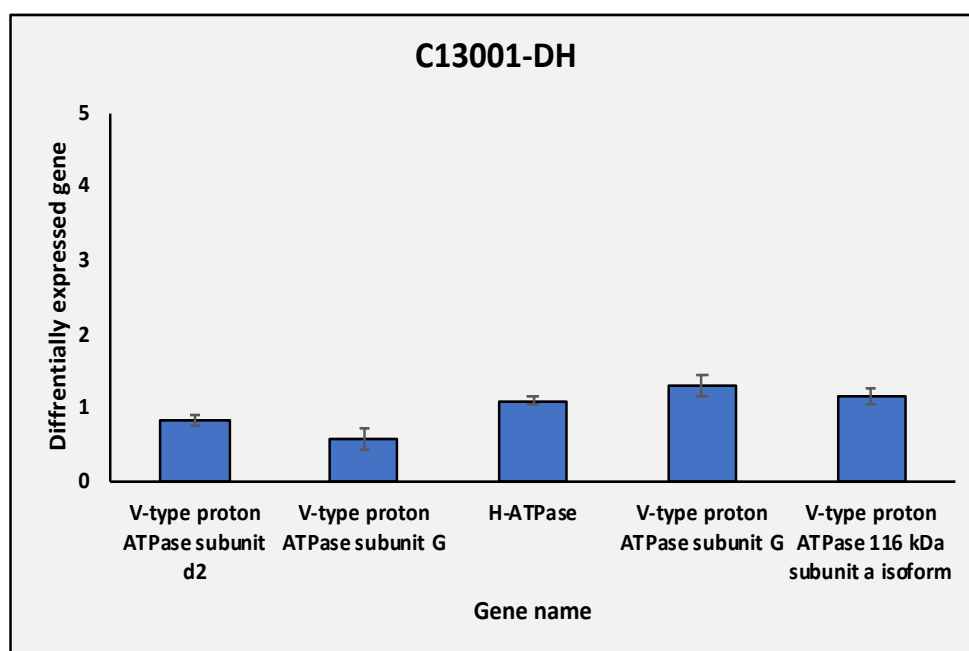


Figure 5.2: Ratio of differentially expressed genes related to vacuolar-type proton pumps ATPases (V-type d2, G subunits and 116kDa subunit an isoform), and proton pump (H-ATPase), under salt stress in salt-treated *B. oleracea* DH genotype as against the salt untreated control. The values represent the ratio between control and treated (n = 3). Error bars are standard error of the mean (SEM).

Similarly, the ratio of differentially expressed genes indicates the fold of expressed genes in treated plant as compared to the untreated control plant is presented in **Figure 5.2**, the expression of vacuolar-type proton pumps was highly expressed in treated plants, i.e., V-type proton ATPase subunit G was 1.28 expressed compared to the control plant. While the two other isoforms of V-type subunit d2 and G showed 0.56 and 0.80 lower expression as compared to the control, which indicates the significant variability of these proton pumps under salt stress condition.

5.2.2 qPCR Analysis

5.2.2.1 Relative expression of Ion membrane transporters genes in twenty-four hours and two-weeks post-salt treatment *B. oleracea* genotypes

Following salt treatment, samples collected 24 hr post-treatment (24 hrpt) and 2 weeks post-treatment (2 wkpt) and qPCR analysis including normalisation of genes against the housekeeping (β -Tubulin) was carried out for both treated and untreated control plants. The results revealed significant physiological and biochemical variations between 24 hrpt and 2 wkpt between the *B. oleracea* genotypes. As expected, some of the membrane ion transporters; Na^+/H^+ antiporters (*NHX1*), Ca^{2+} -ATPases, H^+ -ATPases, *CAX3*, *V-CLC*, V-type proton subunit-G, and V-type subunit-a that were shown to be differentially expressed in the RNA-Seq analysis were also shown to be differentially expressed using qPCR. In this segment of the result, membrane-bound such as Na^+/H^+ antiporters (*NHX1*), calcium-cation exchanger (*CAX3*), and a family member of Ca^{2+} -ATPase (*ECA2*) are presented in **Figures 5.3, 5.4.& 5.5**.

5.2.2.1.1 Na^+/H^+ antiporter (*NHX1*)

The analysis of *NHX1* genes showed significant expression pattern variation between the wild S1 parental lines; *B. oleracea* (C07060-S1), *B. oleracea* (C07079A-S1) and the cultivated rapid cycle founder line DHLS150. The *NHX1* genes showed low expression 24 hrpt in DHLS150 genotype while the wild S1 genotypes, especially *B. oleracea* (C07060-S1) and *B. oleracea* (C07079A-S1) showed higher relative expression when compared to the untreated line (**Figure 5.3**). The doubled haploid genotypes; C13013-DH and C10121-DH both have similar expression of *NHX1* as the parental lines.

Two-weeks post-treatment (2 wk pt), the level of expression of *NHX1* genes were shown to be reduced, this was observed in all the *B. oleracea* genotypes which could further indicates possession of a similar response in relative induction of *NHX1* genes to salt stress conditions in these *B. oleracea* lines. However, C13001-DH genotype showed differences from the other DH genotypes of *B. oleracea* wild S1 extraction. The variation could be as a result of genotype differences and possible effects of allelic recombination that affects the physiological response. The differences in relative gene expression of *NHX1* observed in some *B. oleracea* wild S1 and founder DHLS150 24 hrpt and 2 wkpt could be attributed to high sodium (salt stress) in the growing medium and 2 wkpt could suggests plant's ability to exclude the excess Na^+ might have been reduce (excluded) or properly compartmentalised in the vacuole, which could lead to the lower the expression of *NHX1* (**Figure 5.3**).

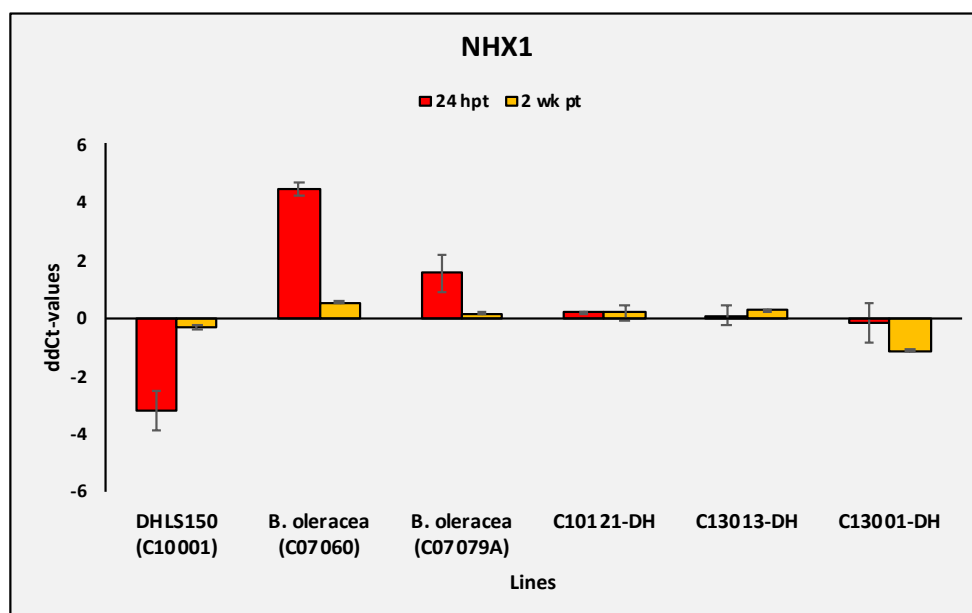


Figure 5.3: Relative expression of Na^+/H^+ antiporter (*NHX1*) genes involved in compartmentalisation of excess Na^+ in response to salt stress in *B. oleracea* lines. Data are expressed as relative gene expression as against the untreated control. Error bars are \pm SEM of three biological replicates and nine technical replicates. **Legend:** Red bars = 24 hr post-salt treatment and Orange bars = two-weeks post-salt treatment.

5.2.2.1.2 Calcium-cation exchanger (*CAX3*) and Ca^{2+} -ATPases (*ECA2*)

A family of $\text{Ca}^{2+}/\text{H}^{+}$ exchanger genes are involved in $\text{Na}^{+}/\text{H}^{+}$ shuttling between the cytoplasm and the vacuole and play a critical role in ensuring the optimal cytoplasmic pH for normal enzymatic reactions. From the result (**Figure 5.4**), *CAX3* showed positive relative expression compared to the untreated control line with significant expression observed in DHLS150, wild S1; *B. oleracea* (C07060-S1 and C07079A-S1) and one of DH line (C13013-DH). The other two DH lines; (C10121-DH) and (C13001-DH), show relative low *CAX3* genes expression. Variation in *CAX3* genes expressional pattern between the parental lines; DHLS150, C07079A-S1 and their derived doubled haploid line C10121-DH could be due to recombination differences. At 24 hrpt, both parent lines showed relative high positive expression when compared to the DH lines as against their controls. Two-weeks post-treatment, the pattern of expression reversed and the parental lines showed lower expression of the *CAX3* genes compared to the DH genotypes except in line C13013-DH, which showed similarities with its parent line (**Figure 5.4**).

Also relative gene expression of an endoplasmic reticulum-bound member of the sub-group of Ca^{2+} -ATPases (*ECA2*) (**Figure 5.4**) investigated showed that *ECA2* genes were highly expressed as against the untreated control 24 hr post-treatment (24 hrpt) in all the *B. oleracea* genotypes with exception of one DH line; C10121-DH, which shows lower expression of *ECA2* and *CAX3* genes. The relative expression of the *ECA2* genes showed to remained low two-weeks post-treatment in all the lines in respect to the control.

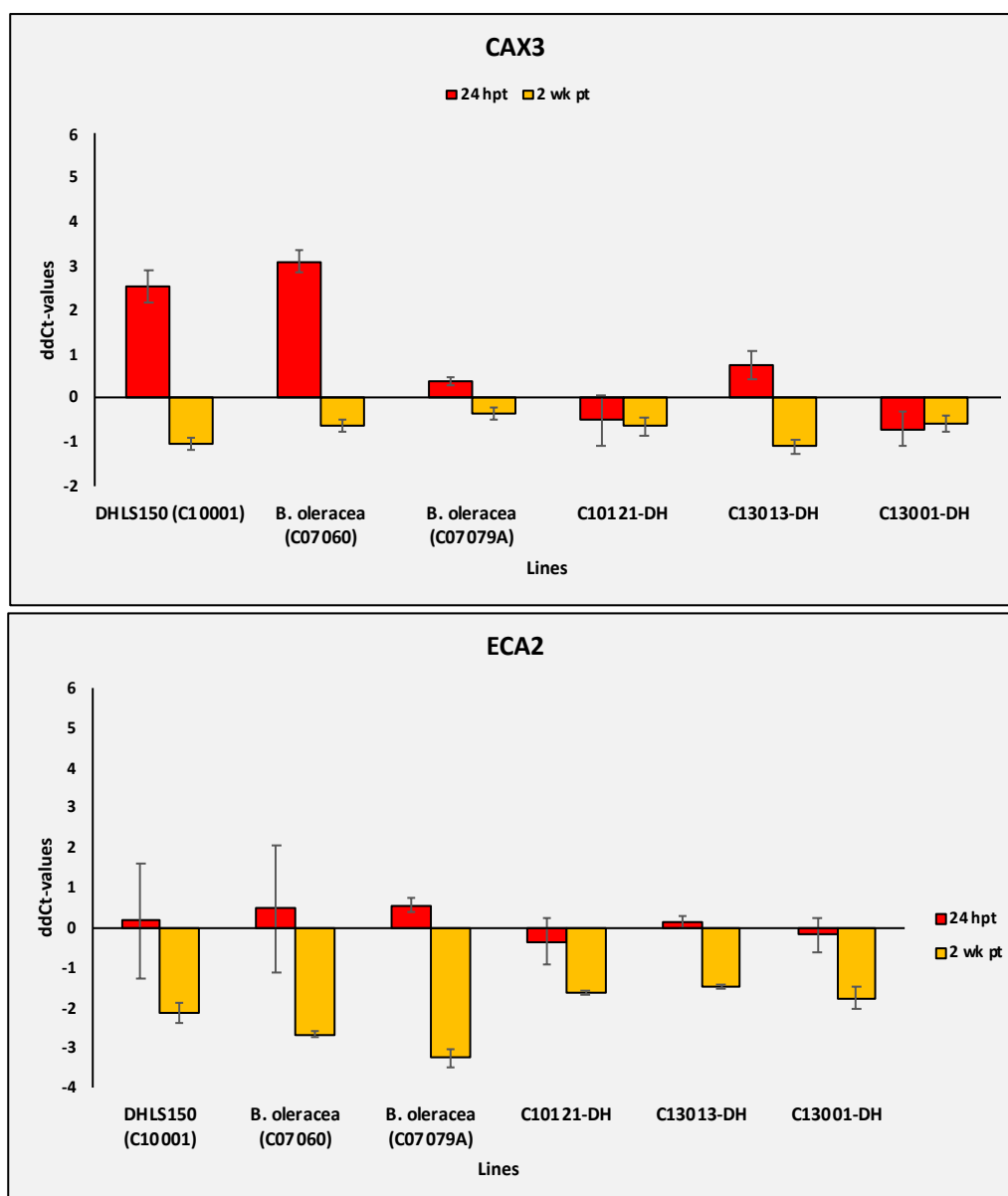


Figure 5.4: Early responses in relative gene expression 24 hr and two-weeks post-salt stress in *B. oleracea* genotypes for calcium-cation exchanger (CAX3) involved in Na^+/H^+ shuttling between the cytoplasm and the vacuole and endoplasmic reticulum-bound Ca^{2+} ATPases (ECA2). Data are expressed as relative gene expression as normalised against the control. Error bars are \pm SEM of three biological replicates and nine technical replicates. **Legend:** Red bars = 24 hr post-salt treatment and Orange bars = two-weeks post-salt treatment.

5.2.2.1.3 High-affinity Potassium Transporter family (*KT9*, *KUP11*, and *KT*)

The high-affinity potassium transporter family (K^+/Na^+ HKT) proteins are bound to plasma membrane, i.e., potassium transporter, (*KT9* & *KT*) and potassium uptake (*KUP11*). These were investigated and result obtained showed that the *KT9* gene was highly expressed in the wild S1 parental lines *B. oleracea* (C07060-S1 and C07079A-S1) respectively 24 hrpt in respect to the control untreated lines (**Figure 5.5**). Furthermore a similar pattern of expression was observed in the *KUP11* gene within the same wild S1 lines (C07060-S1 and C07079A-S1) suggesting both are involved in the response (**Figure 5.5**). The expression fell two-weeks post-treatment. The DHLS150 and DH lines showed low relative expression of *KT9* & *KUP11* genes at both time-points, apart from C13001-DH in which the *KUP11* gene was highly expressed with respect to the control. **Figure 5.5** revealed that the relative expression of *KT* genes was lower in respect to the control in the S1 lines and DH lines. These lines; C07060-S1 and C07079A-S1 were showed to have an appreciable level of K^+/Na^+ ratio 2 wkpt. Expression of these genes could be one of the reasons of the observed higher K^+/Na^+ ratio in these lines (Chapter 4).

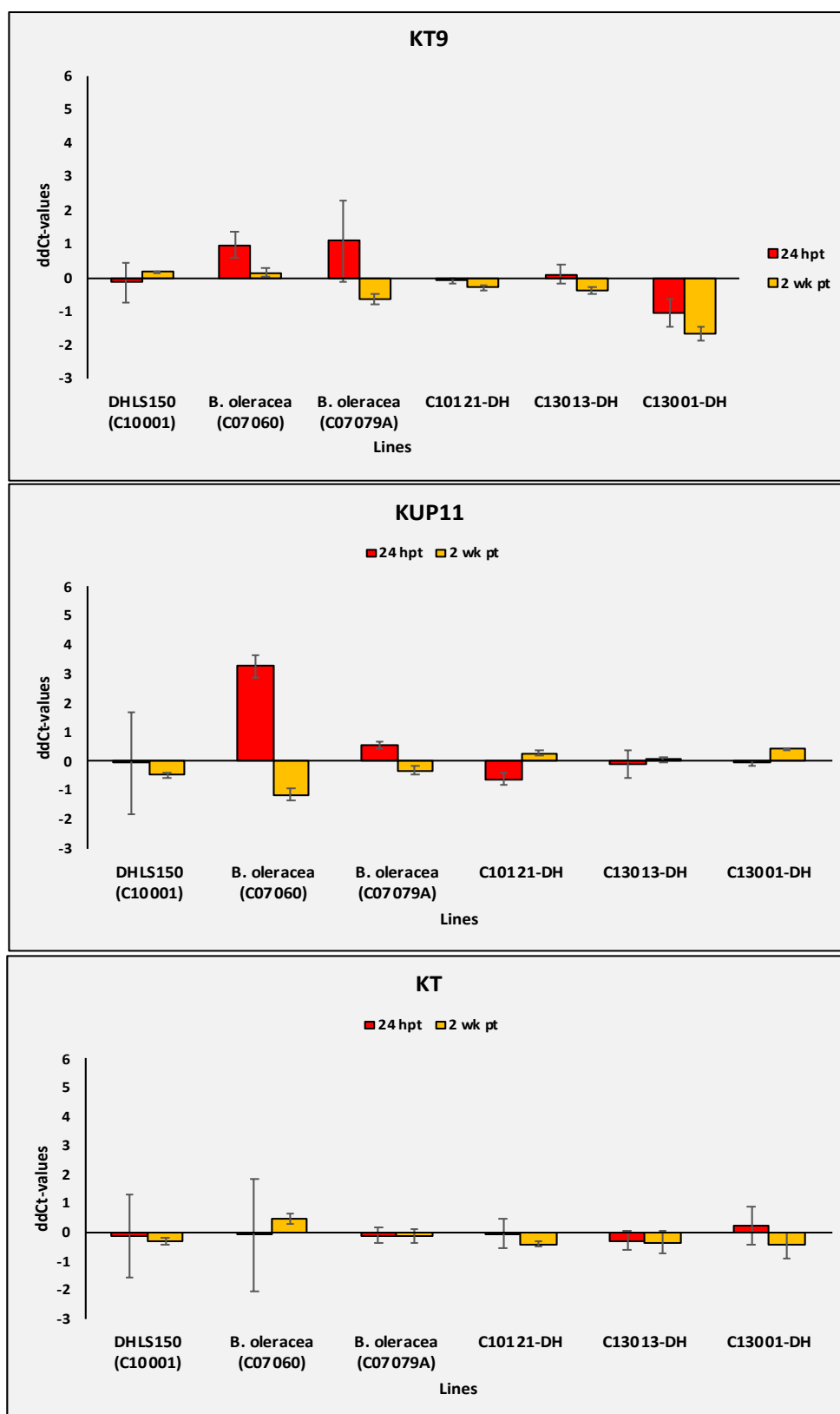


Figure 5.5: Relative expression of genes involved in homeostasis of potassium high-affinity K^+/Na^+ transporter in salt stress in *B. oleracea* genotypes. Data are expressed as relative gene expression as normalised against the control. Error bars are \pm SEM of three biological replicates and nine technical replicates. **Legend:** Red bars = 24 hr post-salt treatment and Orange bars = two-weeks post-salt treatment.

5.2.2.1.4 V-type ATPases (V-type-a1 and G subunits)

In salt stress conditions, active transport of solutes between the cytoplasm and the vacuole remains critical and depend upon the efficiency of proton pumps. Following salt treatment, the expression of *V-type ATPases* genes in *B. oleracea* genotypes was investigated. The result (**Figure 5.6**) showed relative expression of the *V-type-a1* genes was reduced in all the lines. The DH lines and the cultivated rapid cycling parent line DHLS150 showed lower relative expression of *V-type-G* 24 hrpt ., C07060-S1, C07079A-S1 also showed a lower relative expression of *V-type-G* 2 wkpt as compared to the control. In contrast the expression of the *V-type-G* subunit was higher in wild S1 C07060-S1, and C07079A-S when compared to the untreated control. This suggests that this gene varies and could regulate the salt response in these lines.

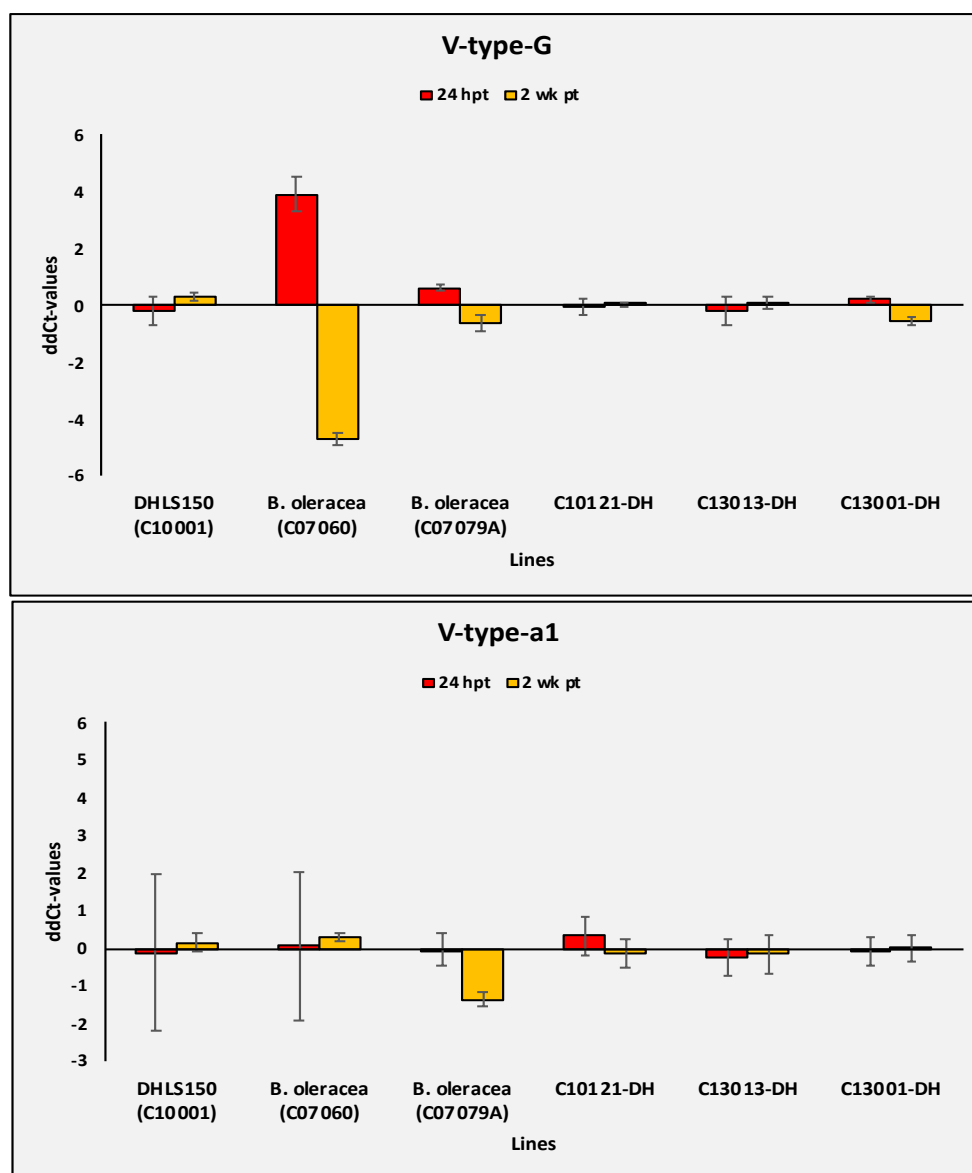


Figure 5.6: Relative expression of vacuolar ATPases a and G subunits that involved in the inward proton gradient that derived Na^+/H^+ for compartmentalisation in vacuole in response to salt stress. Data are expressed as relative gene expression as normalised against the control. Error bars are \pm SEM of three biological replicates and nine technical replicates. **Legend:** Red bars = 24 hr post-salt treatment (24 hrpt) and Orange bars = two-weeks post-salt treatment (2 wkpt).

5.2.2.1.5 Voltage-gated Chloride Channels (V-CLC)

Voltage-gated chloride channels are important channels that regulate the movement of anions and play a critical role in preventing excess chloride from reaching toxic levels in the cytoplasm of a cell. **Figure 5.7** shows the relative expression of the *V-CLC* gene was highly expressed in the wild S1 *B. oleracea* (C07060-S1) and slightly increased expression in the two DH lines; C10121-DH and C13013-DH with respect to the control 24 hrpt. C13001-DH and the other wild S1 *B. oleracea* (C07079A-S1 showed the expression of *V-CLC* genes 24 hrpt to be reduced at this time point. Two-weeks post-treatment, the *V-CLC* gene expression was lower than the control in all the genotypes.

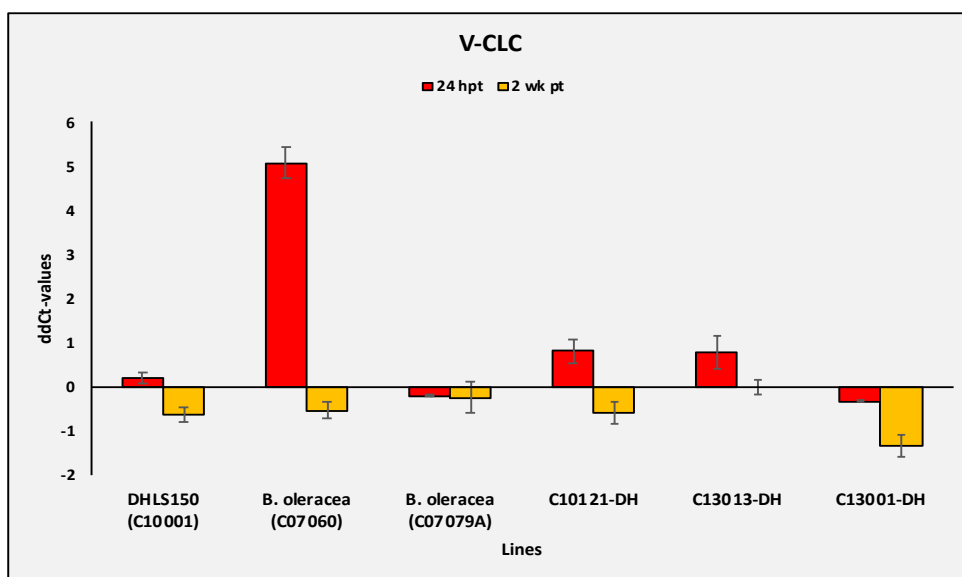


Figure 5.7: Variation in the expression of voltage-gated chloride channels genes that involved for anion transporters and contributes to Cl⁻ movement within plants during salt stress. Data are expressed as relative gene expression as normalised against the control. Error bars are \pm SEM of three biological replicates and nine technical replicates. **Legend:** Red bars = 24 hr post-salt treatment and Orange bars = two-weeks post-salt treatment.

5.3 Discussion

A number of potential salt tolerance genes have been identified, and their function to salt tolerance have been monitored through experiments looking at; the control of salt uptake from roots, regulation pattern of cell influx, long-distance transport mediated through xylem to phloem and compartmentation by plants membrane ion transporters at both cellular as well as tissue levels (Fowler and Colmer 2008). The difference classes relevant to salt tolerance are discussed below.

5.3.1 Na^+/H^+ antiporter (*NHX1*) and V-type-a1 and G subunits

From our results, the level of relative gene expression of the selected plant membrane ion transporters, especially the tonoplast-bound Na^+/H^+ exchangers, *NHX1* genes has shown to be highly expressed in some *B. oleracea* genotypes that were shown to have high K^+/Na^+ ratio and less expressed in others that show low K^+/Na^+ ratio. Many studies have described the vacuolar *NHX1* proteins as group of integral membrane antiporters that catalyse the exchange of cations across tonoplast membrane under the influence of electrochemical gradient generated by the activities of other vacuolar H^+ -ATPases and other proton pumps (Blumwald et al., 2000; Yao et al., 2012; Wang et al., 2013b). In *Arabidopsis*, different isoforms of Na^+/H^+ exchanger (NHX) i.e., *NHX1-4* have been identified to involve in K^+/Na^+ exchange, for H^+ in the vacuole (Bassil et al., 2012; Barragan et al., 2012; McCubbin et al., 2014). Regulation of cytoplasmic pH is critical to plant cell under salinity stress, several studies involving *E. coli*, yeast, plants and animal have suggested that NHX-type antiporters act mechanistically to leak protons in order to fine-tune the luminal pH of specific intracellular compartments (Reguera et al., 2014). High expression of these genes; *NHX1* and V-type-ATPases in *B. oleracea* genotypes 24 hrpt may be a result of excesses of Na^+ and other protons. This could lead to significant membrane depolarisation thus causing

transport of excess ions to the vacuole under an electrochemical gradient established by V-type-ATPases, i.e., V-type-a and G proteins whose genes were shown to be highly expressed in some *B. oleracea* genotypes in response to salt stress. Another important role played by *NHX1* genes, in addition to sequestration, has been associated with cytosolic K^+ uptake into the cell vacuole, as reported by studies of *nhx1/nhx2* knockout (Bassil et al., 2011; Barragan et al., 2012). These observations were further elaborated by work of Liu et al., (2014), which showed that overexpression of *NXHI* genes in transgenic soybean led to a reduction of Na^+ in the shoots and more of K^+ in both roots and shoots, suggesting its role in K^+ homeostasis. Under salt stress conditions, maintaining an appreciable K^+/Na^+ ratio is critical to the plants' survival. Kobayashi et al. (2012), have shown in transgenic rice that lines that showed high expression of *NHX1* genes exhibits higher K^+ content in shoots under salt environment. Importantly, some of our *B. oleracea* genotypes used showed high expression of *NHX1* genes and improved K^+/Na^+ ratio two-weeks post-treatment.

5.3.2 High-affinity Potassium Transporter family (*KT9*, *KUP11*, and *KT*)

High-affinity potassium transporter family are proteins which act both as Na^+/K^+ symporter or uniporter. Studies have proven their presence in the plasma membrane in different plants cell membrane, viz. include wheat, rice, and *Arabidopsis* for its role in salinity tolerance (Waters et al., 2013). Analysis of our result shows that *KT9* and *KUP11* were both expressed twenty-four hours post-salt treatment in some *B. oleracea* genotypes. Potassium transporter genes are classified into a three-member family in plants, namely Shakers K^+ channel, HAK (High-Affinity K)/KUP (K Uptake)/KT (K Transporter) and HKT (High-affinity K Transporter) and are active at the plasma membrane (Wang and Wu, 2013; Very et al., 2014; Shabala and Pottosin, 2014). Under salt stress conditions, plants struggle to

maintain certain level of potassium concentrations to counterbalance the effects of excess sodium to ensure their osmotic potential (Su et al., 2001; Shabala and Pottosin, 2014). Potassium plays a critical role in enzymes activities as a cofactor and in regulation of water movement into the cell to maintain cell turgor. Positive expression of *KT* and *KUP11* in some *B. oleracea* genotypes used could be a counter mechanism to reduce the effects of excess sodium, particularly important in K^+ - Na^+ homeostasis. Studies indicate that *AKT1* in *P. tenuiflora* (*PutAKT1*) was up-regulated under both excess/deficient potassium conditions and under salt conditions, which signify the role of the genes in potassium homeostasis (Ardie et al., 2010). Also, its overexpression has shown to improved salt tolerance in *Arabidopsis* through an increase K^+ uptake (Ardie et al., 2010). The expression of these genes by *B. oleracea* genotypes could be the reason of their improved Na^+/K^+ ratio two-weeks post-treatment.

5.3.3 Ca^{2+}/H^+ - exchangers (*CAX3*) and Ca^{2+} -ATPases (*ECA2*)

Plants Ca^{2+} - ATPases are of two classes; IIA and IIB: i.e., Type IIA are endoplasmic reticulum forms (ECA as ER-type Ca^{2+} -ATPase) and type IIB, autoinhibited Ca^{2+} - ATPase (ACA) (Baxter et al., 2003). The endoplasmic reticulum-bound *ECA2* isoform showed lower relative expression following salt stress in all *B. oleracea* genotypes used. Although no report is available to suggests the key role of *ECA2* genes to salt tolerance, some studies have suggest its possible involvement in potassium and calcium transport (Edelist et al., 2009). For example, using constitutive expression studies, genes related to *KT1*, *KT2*, and *ECA1* have shown to be associated with *SOS1* genes that regulates both potassium and calcium transport in the halophyte *H. paradoxus* (Edelist et al., 2009).

5.3.4 Voltage-gated Chloride Channels (*V-CLC*)

Plants under salinity stress suffer from the effects of excess anions especially chloride (Cl^-). Voltage-gated dependant chloride channels were reported to be located in thylakoids membrane in *Arabidopsis* which function to fine-tune the proton motive force (PMF) and enable plants to adjust variability of light during photosynthesis (Andrei et al., 2015). Under salt stress conditions, excess Cl^- anions tend to affect membrane polarisation and the electrochemical gradient thus, affecting the cytoplasmic pH. Most of the critical enzymes present in thylakoids are of photosynthetic importance, for example, ribulose -1,5-bisphosphate carboxylase/oxygenase (Rubisco), an important regulatory enzyme in Calvin Cycle, was reported to be affected by salinity stress (Bose et al., 2017). Our result showed the relative expression of *V-CLC* genes to be high in some *B. oleracea* genotypes 24 hrpt and lowered expression two-weeks post-treatment. This could suggest its significance in safeguarding the chloroplasts from deleterious effects of excess Cl^- ion under salt stress in these *B. oleracea* genotypes (Bose et al., 2017).

5.4 Summary

Plants salt tolerance has most focussed on selectivity between Na^+ and K^+ to maintain favourable K^+/Na^+ ratios, which require transport systems that involve combinations of membrane transporters that act in different plant parts from roots to shoots. This Chapter reports an investigation which involves validation of some selected ion membrane transporters that are salt stress specific and showed to be expressed 24 hr post-treatment in RNA-Seq data. The study succeeded in the validation of these transporters using qPCR where it shows the relative expression of these genes in different *B. oleracea* genotypes under salt stress at two time-points; 24 hr post-treatment and two-weeks post-treatment. The relative expression of these genes showed variability at different time-points and between genotypes in respect to their untreated control. The data was normalised against housekeeping genes (β -Tubulin and T1P41 see Appendix V). The validation involves the expression of *NHX1*, *CAX3*, *V-CLC*, *ECA2*, *ATPases-a & G* subunits and *KT9*, *KT* and *KUP11* genes. The variation between *B. oleracea* genotypes in the expression of these genes could be as a result of their differences in allelic combination in different segments of DNA and polymorphisms (SNPs). They also form part of active and regulatory sites of different genes and consequently affect responses. Further work is now needed to establish more information on the genetic control and to determine the allelic variation that could be responsible for the observed variation in relative gene expression.

CHAPTER SIX

Allelic Variation and level of Gene Introgression between the *B. oleracea* Genotypes

6.0 Introduction

Natural selection has been the key process responsible for the development of the niches developed between organisms and its environment. In plants breeding, successful selection of genetically superior plants has been associated with the occurrence of genetic variability (Allard, 1960). Genetical variability has been a consequence of the presence of contrasting loci in the genetic constituent under study (Fu et al., 2014). In genetic term, loci are described as either homozygous or heterozygous (Fehr, 1987). Presence of heterozygous loci generally has been implicated as an important factor within breeding programs (Ramalho et al., 2013). This factor was exploited in hybrid progeny that exhibited an enhanced agronomic performance relative to inbred lines. The potential of wildtype species as a source for genetic variation to bring about crop improvement has been known since the early twentieth century (Zamir, 2001; McCouch, 2004). Difficulties related to interspecific breeding, which is associated with cross-incompatibility between the wildtype species and cultivated crop have been suggested. For example, problems like F1-hybrid sterility; infertility of the segregating generations may affect recombination between the chromosomes of the two species; and transfer of trait of interest (reviewed: Alisdair et al., 2006). Wild introgression breeding has been shown to contribute significantly to modern-day varieties despite some challenges (Zamir, 2001). Introgression involves the transfer of one or a number of genes or gene complexes from the plant genetic materials (Haussman et al., 2004).

The level of gene expression can be affected by DNA variation and thus quantitative variants can be detected. For example, naturally occurring allelic diversity in plants has been suggested to be an important cause of phenotypic variation (Doebley and Lukens, 1998; Buckler and Thornsberry, 2002). Induced mutations that eliminate or cause a reduction in gene function, naturally occurring allelic variation modulates gene products and function

and could be an underlying mechanism for quantitative trait variation (Tanksley, 1993; Yano and Sasaki, 1997; Mackay, 2001). Data generated from DNA sequencing has revealed that nucleotide sequence variation is common. For instance, the genome of maize (*Zea mays*) has a higher level of DNA sequence polymorphism than that of humans (Sunyaev et al., 2002; Buckler and Thornsberry, 2002; Ching et al., 2002). The types of allelic variants could be of different, e.g., single nucleotide polymorphisms (SNPs), insertion/deletions (InDels), to large variants runs to several kilobases of DNA fragments (Fu and Dooner, 2002). Such allelic variants are shown to play an important role in gene regulation and affect the expression level. The complex nature and lack of efficient methodology to unravel the relationship of allelic expression differences from changes in a regulatory region that can affect the resultant phenotype have been widely reviewed (Cowles et al., 2002; Glazier et al., 2002).

This chapter aims to investigate allelic variation in the parent *B. oleracea* lines and their derived DH lines and try to determine the level of allelic introgression in the DH lines that could be responsible for the phenotypic variation observed among the *B. oleracea* parental and DH lines in response to salt stress.

This will be achieved by identifying and observing variation within the genes examined in chapter five under salt stress in *B. oleracea* by using data generated by genotype-by-sequencing (GBS).

6.2 Results

6.2.1 Allelic Variation

Following salt stress treatment, transcriptomic profiling performed 24 hr post-treatment has shown the expression of some membrane ion transporters in selected DH lines (**Figures 5.1& 5.2**; Chapter 5). This was followed by further validation by using qPCR (Chapter 5). The genetic variability observed between the parental and DH lines in relative gene expression (Chapter 5) and relative abundance (Appendix VIII) as in respect to the untreated control, which was normalised against house-keeping gene (β -Tubulin). The total DNA of the parents and some DH lines was extracted and analysed using genotype-by-sequencing analysis (GBS). From the GBS analysis, comparison between the parent lines and DH lines showed allelic differences. For example, a close comparisons between some segments of chromosomes in the cultivated founder DHSL150 and that of wild S1 (C07060-S1) (Appendix II **Table S2**), revealed that the arrangement of nucleotide sequence varies in various positions in the same chromosome and within all the chromosomes (Chr.1 to Chr. 9) respectively. Different positions observed have shown substitution, for instance, multiple positions where T/T, alleles appeared in particular position of chromosome in the DHSL150 same alleles was substituted in the wild S1 *B. oleracea* (C07060) with C/C, and this was observed in multiple places where alleles A/A > C/C, and vice versa. This indicates clear variability in the alleles combination of the parent lines that can affect both morphological and physiological response in response to salt stress reported (Chapters 3 and 4) and their genes expression (Chapter 5). Further comparison between the cultivated rapid cycling DHSL150 and wild S1 *B. bourgaei* (C07007-S1) showed differences in variants alleles and such differences appeared repeatedly in multiple positions differentiating C07007-S1 from the DHSL150 parent line (Appendix III **Table S3**). An attempt was also made to compare positions in both parent lines, for example, in chromosome 1 (Chr.1), position of 1,019,546

showed that in cultivated rapid cycling line DHSL150 alleles A/A was substituted in both wild S1 (C07060-S1 and C07007-S1) by C/C alleles. This example was repeated in Chr. 2 position 675,339 A/A alleles in DHSL150 substituted in wild S1 C07060 with T/T alleles while in C07007-S1 position closed to that 675,361 showed C/C variants alleles. These comparisons and examples appeared in many positions in chromosomes (Chr. 1 to Chr. 9) clear indications that alleles recombinational differences could be behind the observed responses between the parent lines and the DH lines.

6.2.2 Introgression in the DH lines

The level of allelic introgression between the cultivated rapid cycling DHSL150 and DH line (C13001) was investigated where positions in the chromosomes were compared in both DHSL150 and C13001-DH (**Table 6.1**). The result showed that significant allelic contribution (Chr.1, 2, 8 and 9) in C13001-DH have been donated by the DHSL150 and was due to a significant introgression that is possibly shared in other wildtype parent lines were observed in chromosomes Chr. 6 and 7 respectively. **Table 6.1**, also showed single nucleotide substitution (SNPs) (in **brown colour text**), observed repeatedly in different chromosome positions, introgression from other wild parent lines (**yellow colour**) and the **uncoloured** segments represent contribution from DHSL150. Likewise, the **blue colour** indicates monomorphic alleles being introgressed into the same chromosome segments from parent lines into the DH line. This could be the underlying factor for the observed similarities in some responses salt stress.

We also compared allelic variants at specific transcripts positions by combining RNA-Seq data that show start- to end- nucleotide base positions of each transcripts and chromosome number its located and GBS data that showed variants alleles in some positions in all the

chromosomes. A comparisons of the positions where transcripts were showed to be located revealed the presence of allelic variants that differed between cultivated rapid cycling DHSL150, and wild S1 (C07060-S1 and C07007-S1) as presented in **Table 6.2**. This suggests possible allelic effects and variation observed in relative gene expression between lines and few positions of allele introgressions at transcripts level were identified in the C13001-DH line **Table 6.2**

Table 6.1: A comparisons of Alleles Introgression in the DH line C13001-DH from the Cultivated DHSL150 and other S1 parent lines

Chr.	Position	DHSL150	Chr.	Position	Chr.	Position	Chr.	Position	Chr.	Position	Chr.	Position	Chr.	Position	Chr.	Position	Chr.	Position																	
C1	12,778,128	T/T	T/T	C2	1,519,749	C/C	C/C	C3	525,697	C/T	T/T	C4	2,290,503	T/T	T/T	C5	1,139,902	G/G	A/A	C6	668,916	C/T	C/C	C7	1,178,931	T/G	T/G	C8	1,452,146	T/T	T/C	C9	2,157,116	C/C	C/T
C1	12,778,144	T/T	T/T	C2	1,519,773	T/T	C/C	C3	525,720	T/C	C/C	C4	2,290,520	T/T	T/T	C5	1,150,273	G/G	T/T	C6	5,550,498	T/T	T/T	C7	1,178,956	C/T	C/T	C8	1,452,151	T/T	T/T	C9	9,954,476	T/A	T/A
C1	12,808,630	T/T	T/T	C2	1,570,016	T/T	C/C	C3	525,740	T/G	T/G	C4	2,314,923	T/T	T/T	C5	1,150,279	G/G	A/A	C6	5,550,585	T/T	T/T	C7	1,178,972	A/C	A/C	C8	1,452,164	T/T	T/C	C9	9,954,487	T/C	C/C
C1	12,919,117	T/T	T/T	C2	1,570,143	C/C	C/C	C3	525,767	G/C	G/C	C4	2,326,724	T/T	T/T	C5	1,150,782	C/C	T/T	C6	5,550,590	T/T	T/T	C7	1,178,991	A/T	A/T	C8	1,452,165	T/T	T/C	C9	9,954,522	G/T	G/G
C1	13,244,706	A/C	A/C	C2	1,658,484	A/A	G/G	C3	1,727,968	A/G	G/G	C4	2,357,158	T/T	T/T	C5	1,150,830	G/G	A/A	C6	11,516,161	C/C	T/T	C7	7,752,294	T/T	T/T	C8	7,917,220	T/C	C/C	C9	9,954,536	C/T	C/T
C1	13,244,719	A/G	A/G	C2	1,658,488	A/A	C/C	C3	1,743,337	C/C	C/C	C4	4,989,297	G/A	G/A	C5	1,150,977	T/T	A/A	C6	11,516,171	C/C	A/A	C7	7,752,384	T/T	T/T	C8	37,654,684	G/G	G/C	C9	38,522,964	C/A	C/A
C1	13,244,734	T/G	T/G	C2	1,658,509	G/G	G/G	C3	1,743,678	T/T	T/T	C4	4,989,307	G/A	G/A	C5	1,173,909	A/A	T/T	C6	11,516,200	T/T	C/C	C7	7,784,954	T/T	T/T	C8	37,654,696	A/A	G/A	C9	38,522,978	G/T	G/T
C1	13,392,095	C/T	C/T	C2	1,663,064	T/T	C/C	C3	1,853,124	A/A	A/A	C4	4,989,358	C/G	C/G	C5	1,175,959	T/T	C/C	C6	12,850,536	C/A	C/C	C7	10,786,161	C/C	C/C	C8	37,654,726	A/A	A/G	C9	47,910,542	C/C	C/C
C1	14,230,334	T/T	T/T	C2	1,675,215	T/T	C/C	C3	1,853,129	T/T	T/T	C4	5,027,007	C/C	C/C	C5	1,248,191	C/C	T/T	C6	13,930,631	C/T	C/C	C7	10,786,184	C/C	C/C	C8	40,607,704	G/G	G/G	C9	47,929,614	G/G	G/G
C1	14,230,337	T/T	T/T	C2	1,678,594	C/C	C/C	C3	1,853,153	T/T	T/T	C4	5,057,711	T/T	C/T	C5	1,464,888	C/A	C/C	C6	18,452,501	T/T	C/T	C7	10,792,499	C/C	C/C	C8	40,607,829	G/G	G/G	C9	47,929,642	T/T	T/T
C1	14,230,353	T/T	T/T	C2	1,689,290	G/G	T/T	C3	1,869,050	T/T	T/T	C4	5,057,714	T/T	T/C	C5	1,464,894	G/A	A/A	C6	31,840,368	A/G	A/A	C7	10,810,089	C/C	C/C	C8	40,675,250	G/G	G/G	C9	47,929,644	A/A	A/A
C1	14,230,363	T/T	T/T	C2	1,689,311	T/T	C/C	C3	1,912,212	C/C	T/T	C4	5,108,928	T/T	T/T	C5	1,464,906	C/C	C/C	C6	33,616,485	C/G	C/G	C7	10,810,101	T/C	T/C	C8	40,675,322	A/A	A/A	C9	47,945,582	C/C	C/C
C1	14,565,988	C/C	C/C	C2	1,689,350	A/A	T/T	C3	2,976,415	G/G	C/C	C4	18,146,220	C/A	C/A	C5	1,464,912	G/A	G/C	C6	33,617,212	A/G	A/G	C7	17,622,547	A/T	A/A	C8	40,677,578	T/T	T/T	C9	47,977,854	C/C	C/C
C1	14,566,117	C/C	C/C	C2	1,806,627	T/T	T/T	C3	2,976,600	C/C	C/C	C4	18,249,575	A/G	A/G	C5	1,882,439	T/T	T/T	C6	38,490,202	C/A	C/A	C7	20,455,749	C/T	C/C	C8	40,726,097	T/T	T/T	C9	47,977,859	A/A	A/A
C1	14,566,133	T/T	T/T	C2	1,806,639	G/G	T/T	C3	3,036,810	G/G	T/T	C4	19,787,101	G/A	G/G	C5	1,886,551	T/T	T/T	C6	38,490,225	C/C	T/T	C7	35,143,830	C/T	C/T	C8	40,726,137	T/T	T/T	C9	47,984,281	A/A	A/A
C1	14,570,527	T/T	T/T	C2	1,806,713	C/C	T/T	C3	3,047,975	T/T	T/T	C4	19,802,244	G/T	G/T	C5	1,886,561	T/T	T/T	C6	38,493,446	T/T	C/C	C7	35,143,839	C/G	C/G	C8	40,726,209	C/C	C/C	C9	47,984,299	C/C	C/C
C1	14,570,577	T/T	T/T	C2	1,809,217	G/G	C/C	C3	3,048,030	T/T	T/T	C4	19,802,255	C/A	C/A	C5	1,886,566	T/T	T/T	C6	38,493,540	C/C	C/C	C7	35,143,842	G/A	G/A	C8	40,726,227	A/A	A/A	C9	48,011,262	C/C	C/C
C1	14,570,637	C/C	C/C	C2	1,809,293	A/A	T/T	C3	3,050,304	G/G	T/T	C4	19,888,677	A/A	A/A	C5	3,358,619	G/A	G/G	C6	38,493,636	A/A	T/T	C7	35,143,854	T/C	T/C	C8	40,726,293	A/A	A/A	C9	48,011,278	T/T	T/T
C1	14,570,662	C/C	C/C	C2	1,826,117	A/A	A/A	C3	3,050,345	G/G	A/A	C4	19,916,780	T/T	T/T	C5	3,358,628	A/C	A/C	C6	38,499,410	C/C	C/C	C7	42,057,564	G/G	A/A	C8	40,726,455	T/T	T/T	C9	48,089,796	T/T	T/T
C1	14,690,287	G/G	G/G	C2	1,860,888	C/C	G/G	C3	3,050,441	T/T	A/A	C4	20,064,456	A/A	A/A	C5	4,027,925	C/C	C/C	C6	38,499,440	T/T	G/G	C7	42,139,168	G/G	A/A	C8	40,733,163	G/G	G/G	C9	48,089,817	T/T	T/T
C1	14,775,420	T/T	T/T	C2	1,931,177	C/C	C/C	C3	3,547,025	T/T	C/C	C4	20,175,960	C/T	C/T	C5	4,027,961	C/C	C/C	C6	38,527,168	A/A	G/G	C7	42,139,213	G/G	A/A	C8	40,733,188	C/C	C/C	C9	48,089,844	T/T	T/T
C1	14,775,426	T/T	T/T	C2	1,931,195	T/T	C/C	C3	3,575,147	T/T	T/T	C4	20,175,973	C/T	C/T	C5	4,027,970	C/C	C/C	C6	38,578,260	A/A	G/G	C7	42,139,264	T/T	C/A	C8	40,733,193	G/G	G/G	C9	48,153,478	C/C	C/C
C1	14,887,712	T/T	T/T	C2	4,034,504	C/C	C/C	C3	3,581,417	T/T	T/T	C4	20,177,640	C/T	C/T	C5	4,028,575	C/C	C/C	C6	38,578,297	A/C	A/A	C7	42,139,305	A/A	G/G	C8	40,733,214	C/C	C/C	C9	48,155,731	C/C	C/C
C1	14,897,514	T/T	T/T	C2	4,062,700	C/C	C/C	C3	3,581,429	T/T	T/T	C4	20,317,039	G/A	A/G	C5	9,032,865	A/G	A/G	C6	38,584,827	T/T	T/T	C7	42,304,467	A/A	C/C	C8	40,776,265	G/G	G/G	C9	48,229,808	C/C	C/C
C1	14,900,379	C/A	C/C	C2	4,062,730	T/T	C/C	C3	3,581,453	T/T	T/T	C4	20,317,079	C/T	C/T	C5	9,032,890	C/T	C/T	C6	38,584,828	A/A	C/A	C7	42,322,943	C/C	G/G	C8	40,776,269	G/G	G/G	C9	48,282,752	C/C	C/C
C1	14,928,901	A/A	A/A	C2	4,067,421	T/T	T/T	C3	26,436,020	T/T	T/T	C4	20,798,837	A/T	A/A	C5	9,137,773	G/T	G/T	C6	38,600,672	T/T	C/C	C7	42,412,976	A/A	C/C	C8	40,780,307	G/G	G/G	C9	51,305,490	T/T	T/T
C1	14,928,994	C/T	C/T	C2	5,376,130	C/C	C/C	C3	26,436,459	T/T	T/T	C4	26,375,457	G/G	G/G	C5	16,247,592	T/T	T/T	C6	38,601,265	T/T	A/A	C7	42,413,112	G/G	T/T	C8	40,780,310	T/T	T/T	C9	51,305,492	A/A	A/A
C1	14,928,999	T/C	T/C	C2	5,376,137	C/C	C/C	C3	26,436,488	T/T	T/T	C4	26,375,472	C/G	G/G	C5	16,247,603	T/T	T/T	C6	38,601,516	G/G	A/A	C7	42,587,471	A/A	T/T	C8	40,780,337	T/T	T/T	C9	51,323,388	T/T	T/T
C1	18,176,385	C/C	C/C	C2	5,376,168	C/C	C/C	C3	26,437,539	T/T	T/T	C4	28,611,739	T/T	T/T	C5	16,248,743	T/T	T/T	C6	38,601,576	C/C	G/G	C7	42,587,537	T/T	C/C	C8	40,782,341	G/G	G/G	C9	51,323,404	T/T	T/T
C1	18,177,422	C/C	C/C	C2	5,376,189	G/G	A/A	C3	26,438,277	T/T	T/T	C4	28,630,315	T/T	T/T	C5	16,292,336	T/T	T/T	C6	38,601,577	A/A	G/G	C7	42,598,382	T/G	T/T	C8	41,392,747	C/T	C/T	C9	51,323,557	T/T	T/T
C1	18,184,466	C/C	C/C	C2	5,376,280	T/T	T/T	C3	26,438,315	C/C	C/C	C4	28,646,133	T/T	T/T	C5	16,292,413	T/T	T/T	C6	38,601,594	G/A	G/A	C7	42,602,533	G/G	T/T	C8	41,392,753	G/A	G/A	C9	51,342,443	T/T	T/T
C1	18,234,998	C/C	C/C	C2	5,376,304	T/T	C/C	C3	26,438,438	T/T	T/T	C4	28,651,848	T/T	T/T	C5	20,263,167	C/A	C/C	C6	38,601,607	G/G	C/C	C7	42,603,503	G/G	T/T	C8	41,392,843	G/A	G/A	C9	51,342,477	T/T	T/T
C1	25,591,595	C/T	C/T	C2	5,382,474	A/A	A/A	C3	26,467,345	T/T	T/T	C4	28,651,927	T/T	T/T	C5	29,200,864	C/T	T/T	C6	38,601,620	G/G	A/A	C7	42,603,523	A/A	T/T	C8	41,392,891	C/A	C/A	C9	51,342,522	C/C	C/C
C1	25,591,601	C/T	C/T	C2	5,382,527	T/T	T/T	C3	26,597,168	A/G	A/G	C4	28,770,467	T/T	T/T	C5	32,948,404	C/T	C/T	C6	38,611,726	A/A	C/C	C7	42,692,956	A/A	G/G	C8	41,392,906	T/A	T/T	C9	51,342,563	T/T	T/T
C1	25,899,042	T/C	C/C	C2	5,384,780	G/G	G/G	C3	26,597,189	A/T	A/T	C4	42,956,967	T/T	C/T	C5	32,949,529	G/G	G/G	C6	38,617,419	A/A	C/C	C7	42,708,380	A/A	G/G	C8	41,419,201	C/C	C/C	C9	51,358,271	T/T	T/T
C1	25,952,193	T/A	T/T	C2	5,385,535	T/T	T/T	C3	26,597,195	C/T	C/T	C4	45,904,240	T/C	A/A	C5	32,949,539	A/G	A/G	C6	38,618,373	T/T	C/C	C7	42,708,449	T/T	G/C	C8	41,419,220	C/C	C/C	C9	51,374,427	C/C	C/C
C1	26,151,205	T/T	T/T	C2	5,385,568	T/T	T/T	C3	29,105,463	C/C	C/C	C4	45,904,261	C/A	C/A	C5	32,950,709	T/C	T/C	C6	38,704,916	G/G	A/A	C7	42,876,601	T/T	C/C	C8	41,446,514	C/C	C/C	C9	51,374,430	T/T	T/T
C1	26,153,682	T/T	T/T	C2	5,385,577	T/T	T/T	C3	29,118,241	C/C	C/C	C4	45,904,274	C/C	C/T	C5	35,305,466	T/T	T/T	C6	38,772,600	C/C	T/T	C7	42,876,607	G/G	C/C	C8	41,446,547	C/C	C/C	C9	51,374,673	G/G	G/G
C1	26,198,341	T/T	T/T	C2	5,385,599	T/T	T/T	C3	29,118,279	C/C	C/C	C4	45,904,277	A/C	A/C	C5	35,306,159	T/T	T/T	C6	38,929,073	G/G	T/T	C7	42,876,635	C/C	A/A	C8	41,448,274	A/A	A/A	C9	51,374,757	C/C	C/C
C1	26,238,845	T/T	T/T	C2	35,020,957	G/A	G/A	C3	29,118,280	C/C	C/C	C4	51,365,287	T/T	T/T	C5	35,306,180	T/T	T/T	C6	38,929,206	C/C	T/T	C7	42,877,087	A/A	T/T	C8	41,448,343	T/T	T/T	C9	51,375,364	T/T	T/T
C1	26,268,633	A/A	A/A	C2	35,021,030	G/C	G/C	C3	29,118,312	C/C	C/C	C4	51,365,300	T/T																					

Table 6.2: Differences in nucleotides at various transcripts position and introgression in DH line from the wild parent line

		Name									
		. oleracea		B. oleracea		B. bourgeai					
Chr. No.	Chr.	Position	To1000	DHSL150	C07060-S1	C07007-S1	C13001-I	Gene ID	Start (bp)	End (bp)	Gene name
C1		7,746,149		T/T	A/A						
		8,154,945		T/T	A/A			Bo1g022080	8,072,815	8,076,088	KT9
		8,003,596	G	G/G		T/T					
		8,154,695	C	C/C		G/G					
C1		43,668,828	T	A/A	T/T	T/T		Bo1g158860	43,713,959	43,714,450	Vitype-G
		43,722,251	T	C/C	T/T	T/T					
C2		6,645,829		G/G	T/T			Bo2g024320	6,673,393	6,676,356	V-type-a
		6,782,085		G/G	A/A						
		6,645,814	T	T/T		C/C					
		6,682,898	G	G/G		T/T					
C3		21,704,721		A/A	T/T			Bo3g055180	21,774,777	21,775,402	V-type-G
		21,877,988		A/A	C/C						
		21,773,035	G	G/G		C/C					
		21,884,858	A	A/A		G/G					
C4		1,354,904		A/A	G/G						
		1,359,488		A/A	G/G			Bo4g012670	1,353,531	1,360,063	KT
		1,111,042	G	G/G		A/A					
		1,369,050	T	T/T		C/C					
C4		8,528,513		C/C	T/T						
		8,529,098		C/C	T/T						
		8,529,129		C/C	T/T						
		8,529,134		T/T	C/C			Bo4g039050	8,527,051	8,530,274	KUP11
		8,552,082		A/A	G/G						
		8,473,828	G	G/G		A/A					
C5		8,622,918	C	C/C		G/G					
		40,570,121		C/C	T/T						
		40,672,868		C/C	T/T			Bo5g131740	40,620,196	40,622,124	CAX3
		40,570,121	C	C/C		T/T					
C5		40,740,571	C	A/A		C/C					
		46,654,387		T/T	C/C			Bo5g155190	46,731,498	46,732,608	V-type-G
		46,712,526	A	G/G		A/A					
C7		46,832,735	A	A/A		C/C					
		43,058,058	C	T/T	C/C		C/C	Bo7g109030	43,064,941	43,065,512	V-type-G
		43,094,940	T	T/T	G/G		G/G				
C7		47,425,896		T/T	A/A						
		47,626,895		C/C	T/T			Bo7g11864	47,615,558	47,619,804	CAX3
		47,542,369	C	G/G		C/C	G/G				
		47,630,058	A	G/G		A/A	T/T				
C8		9,920,732		A/A	G/G			Bo8g030800	10024031	10027008	Voltage-CLC
		10,314,816		G/G	T/T						
		9,934,618	C	T/T		C/C					
C8		10,855,681	A	A/A		C/C					
		31,084,096		G/G	A/A			Bo8g091730	31,084,759	31,089,409	Ca-transpoter
		31,206,415		G/G	T/T						
		31,041,194	A	C/C		A/A					
C9		31,095,711	G	A/A		G/G					
		510,525		A/A	G/G			Bo9g003910	514674	519143	ECA2
		515,690		T/T	G/G	G/G					
		534,744		C/C	T/T	T/T					
		2,920,600		A/A	T/T						
C9		3,051,301		C/C	T/T						
		2,935,281	C	C/C		T/T		Bo9g010200	2,940,449	2,943,704	NHX1
		3,069,453	C	C/C		T/T					

* **Note:** colours used indicate differences in nucleotide base at different chromosome positions where the genes are located. We observed two introgression positions in the DH line Chr. 7 for *V-type-G* and *CAX3* transcripts.

6.2.3 Discussion

The allelic variation between the *B. oleracea* cultivated DHSL150, wild S1, and introgressed DH lines have been investigated and from the results, it becomes clear that allele variants occur in both parental lines and a DH line. The presence of allelic variants have been shown to play a critical role in gene regulation and could affect the level of gene expression or the proteins produced (Mackay, 2001). The sequence data studied reveals the existence of variants insertion, inversion, and substitutions of a particular allele, in close proximity in the parental chromosomes. This indicates genetic diversity between them and could be the source of the observed differences in response to salt shock treatment and gene expression and regulation as allelic variation has been shown to modulates gene response and its relative abundance (Mackay, 2001).

Hybridisation between two plant species is known to affect gene expression due to genetic variation (Adam, 2007). Up- and downregulation of gene expression in hybrids compared to their parents have been reported in interspecific and intraspecific hybridisation between cultivars in diploid and triploid maize hybrids (Hegarty et al., 2005; Auger et al., 2005; Guo et al., 2006; Stupar and Springer, 2006; Meyer et al., 2007). Furthermore, hybridisation has shown to affect allele frequencies which can modulate gene expression. Allele-specific regulation in response to environment and abiotic stress in maize hybrids have shown the un-equivalent function of the parental alleles in the hybrid (Guo et al., 2004). This suggests that variation between the parents and hybrids DH lines observed could be as a result of the un-equivalent function of the parental alleles and linkage disequilibrium could affect the level of introgression, and functionally of important variants (Knight, 2004).

Variation in nucleotide bases (substitutions) at various DNA positions and at different positions of genes in the chromosomes could be attributed to the variation in relative gene expression, morphological and physiological responses between the parent lines and DH lines. Studies on how parent-of-origin affect gene expression by using reciprocal crosses between two different maize lines showed that some genes were expressed differently exhibiting parental effects on gene expression (Stupar and Springer, 2006). Hybridisation has been suggested to be a source of loss of genetic imprint. A study by Josefsson et al. (2006), reported a loss of imprinting of two genes and attributed that to the effects of hybridisation between tetraploid *A. thaliana* and diploid *A. arenosa*.

6.2.4 Summary

This chapter studied allelic variation and defined introgression between the parent lines and the DH lines. The aim was to unravel the relationships and to establish linkages in the observed response to salt stress in *B. oleracea*. The chapter utilises information obtained from RNA-Seq and that of genotype-by-sequencing to specifically compare variants allele positions in the chromosome. A comparison between the parent lines; DHSL150 and Wild S1 lines has shown clear differences existed. Regions that are not conserved could be responsible for the observed morphological, physiological and gene expressional responses. Specific introgression from the wildtype material can be introgressed into DH lines. Integration and expression of these traits by the DH lines show good genetic background in the lines that enable expression. Further work to establish the number of SNPs and their positions especially between the parent lines; DHLS150 and wild S1 and positions could lead to identification of more introgression, which is good for breeding diversity into *B. oleracea* vegetables.

CHAPTER SEVEN

General Discussion And Future Work

7.0 General Discussion

Food security remains of global concern and is on the concurrent list of concerns of many developed and developing countries. A report from the Food and Agriculture Organisation (FAO) highlighted significant challenges in the areas of production, indicating 70% more food would be needed to feed 2.3 billion additional population by the year 2050. Brassicas are essential sources of food and form a major part of the human diet and are ranked third after cereals and legumes. Apart from the oilseeds (mustard seed, oilseed rape), the brassicas are grown for food; such crops includes kohlrabi, turnips, cauliflower, broccoli, cabbages, and Brussels sprout. Brassica vegetables remain a source of essential bioorganic elements that have both nutritional and medicinal properties (Beecher, 1994; Carvalhoa et al., 2006). They are rich in soluble fibers and vitamins (Divisi et al., 2006). Members are also good sources of anticancer elements such as 3, 3' diindolylmethane, sulforaphane, and selenium (Finley et al., 2005; Banerjee et al., 2012). The decline in food productivity can be attributed to various environmental abiotic factors. For example, salinity stress is one of the major factors that affect plants growth and productivity. Efforts toward improving crops tolerance to salt stress utilizing both conventional and advance genetic breeding program have so far made significant progress. In part, this is due to the genetic complexity of salt tolerance which makes the task more challenging and research driven.

Therefore, the aim of this thesis was to investigate salt resilience using Brassica genetic resources, including wild *B. oleracea* C-genome genotypes and a cultivated accession DHSL150, Wild S1 and doubled haploid lines derived from a cross between wild and domesticated lines were also used. All lines were exposed to salt shock stress. It is believed that this study will increase our understanding and capacity to breed for improved response to salinity stress. Furthermore, the objective has been to find the level of natural variation

and gene flow between the parental and doubled haploid lines conveying tolerance to a salt stress environment. This was achieved by combining evaluation of some phenotypic traits including plant growth, plant fresh/dry weights, leaf fresh/dry weights and leaf area, and physiological through the estimation of leaf mineral content, with transcriptomic profiles and a specific focus on membrane ion transporters, qPCR and genotype-by-sequencing to understand the cause of variation in response to salt stress.

Growth analysis is a fundamental characteristic to study plant's response to environmental stress (Mane et al., 2010). The effects of salt stress on plant growth was investigated in Chapter 3 and the phenotypic traits measured have shown significant variation among the *B. oleracea* genotypes tested. The physiology and morphological responses to salt stress vary among plants such have been exhibited by halophytes and less tolerant plants (Flower, 2004, Mane et al., 2010). The current study has illustrated that salt tolerance among the *B. oleracea* genotypes varies, the cultivated founder DHSL150 has shown to be more susceptibility in morphological traits as those the wild S1 parents (**Figures 3.2a; 3.3 and 3.4**). Some of the wild S1 and the doubled haploid lines have shown improve plant height, plant fresh and dry weights and leaf area (**Figures 3.2a; 3.3; 3.4 and 3.8a**) For example, salt stress causes plant growth reduction by affecting cell expansion, stomatal closure and photosynthesis, these are the important biochemical pathways by which plants obtain food material for growth (Kojo, 2004). Also, oxidative stress due to the excess salt has shown to contribute to the deleterious effects of salt and causes significant growth reductions in plants (Hemandez et al., 1999). Furthermore, the effects of salt stress reduce water concentrations of leaves and shoot thus causing a reduction in biomass accumulation. In the current study, it has been shown that leaf area is an important indicator for improved net CO₂ assimilation and an increase in leaf thickness was observed in wild S1 and doubled haploid lines. This

perhaps indicates allelic flow between the parents and derived double haploid lines from the F1-hybrids materials.

Similarly, salinity stress has shown to effects ion homeostasis and causes an increase in Na^+ level. Sodium competes with K^+ thereby decreasing the availability of K^+ for optimal cell turgor and enzymatic reactions. In Chapter 4, the effects of salt stress on mineral content in *B. oleracea* genotypes, wild S1, DHSL150 and doubled haploid lines was investigated. The result demonstrates that leaf Na^+ content increased following salt stress 24 hr post-salt treatment and this is supported by a study of Rezaei et al. (2017), which shows significant differences in *B. napus* L. cultivars. Increasing Na^+ content in brassica cultivars has been reported by studies of Rameeh et al. (2004) and Tanveer et al. (2002). The observed variation in leaf Na^+ content among the *B. oleracea* genotypes could be attributed to the differences in physiology and molecular mechanisms which are subject to genetic control. The current study also shows that the *B. oleracea* genotypes were able to reduce the level of leaf Na^+ content two-weeks post-treatment. Zhu (2003), reported that Na^+ cytotoxicity led to a replacement of K^+ by Na^+ in the biochemical process and disturbed amino acids positions and their protein function. Moreover, Na^+ led to an imbalance in metabolic, osmotic stress and nutritional deficiency. It was observed that the wild S1 and doubled haploid *B. oleracea* genotypes effectively exclude Na^+ through exclusion mechanism and some show their ability to compartmentalise Na^+ , making them more effective under salty conditions through the management of Na^+ ions in their leaves or shoots so they could grow. The current study also reported the K^+/Na^+ ratios as it relates to salt stress in *B. oleracea* genotypes, which shows a reduction in K^+/Na^+ ratio 24 hr post-salt treatment. This observation is corroborated by a study on *B. napus* L. genotypes under salt stress, which also reported K^+/Na^+ reduction following salt stress (Rezaei et al., 2017). Reduction in

K^+/Na^+ ratio was earlier reported in brassica cultivars (Ashraf, 2004), green bean (Yasar et al., 2006), wheat (Hu et al., 2006) and legumes (Amador et al., 2007). An improvement in K^+/Na^+ ratio has been associated with salt tolerance in plants (Maathius and Amtmann, 1999).

Ionic stress is perhaps the most serious components of salt stress and brought about by excess Na^+ accumulation in especially at the aerial parts of plants (Munns and Tester 2008). Na^+ ions interfere with K^+ homeostasis and given its involvement in a number of metabolic processes, thereby hinders the plant's ability to maintain a balanced cytosolic K^+/Na^+ ratio, which is more important to salt tolerance mechanism. To achieve balance homeostasis, it requires the activity of K^+ transporters and/or channels. The observed differences between *B. oleracea* genotypes against salt stress could be a result of differences in both structural and functional proteins that act as enzymes and membrane channels responsible for these processes.

In Chapter 5, relative gene expression of some selected ion membrane transporters involved in Na^+ and K^+ homeostasis were investigated to further understand the responses between the wild S1, DHSL150 and doubled haploids in salt stress conditions. In salt stress, maintenance of stable membrane potentials is critical for salt stress tolerance as excess Na^+ induces depolarization of the membrane causing electrolytes to shift. In addition, novel regulators of ion membrane transporters such as Na^+/H^+ antiporters (NHX1) genes, vacuolar proton pumps that create an electrochemical gradient, V-type and H^+ -ATPases and high-affinity potassium transporter family (HAK) such as KUP11 and KT9 were investigated. The *B. oleracea* genotypes have shown variation in the expression of these ion membrane transporters under salt stress. It was reported in chapter 4 (Table 4.3) that wild S1 lines have an improved K^+/Na^+ ratio which could be related to possibly their high relative expression

of these membrane transporters such as *NHX1*, *KT9*, and *KUP11* as demonstrated in chapter 5 (**Figures 5.3 & 5.5**). This was observed in the wild S1 *B. oleracea* (C06070-S1 & C07079A-S1) (**Figures 5.3 & 5.5**) and less relative expression in C13013-DH and C10121-DH which showed a relative increase in K^+/Na^+ ratio two-weeks post-salt treatment as indicated in chapter 4 (**Table 4.3**). However, the relative gene expression of the genes significant vary, with some showed high expression values such in the wild S1 lines as compared to the DH lines (**Figures 5.3 & 5.5**). The relative gene expression in the cultivated DHSL150 and a DH line C13001-DH have been relatively lower as compared to the untreated control and both showed lower K^+/Na^+ ratio two-weeks post-salt treatment as also indicated in chapter 4 (**Table 4.3**) and chapter 5 (**Figures 5.3 & 5.5**). Other transporters investigated showed similar expression pattern between the wild S1, DH and cultivated DHSL150 (**Figures 5.6 & 5.7**).

Chapter six investigates the effects of allelic variation and polymorphism on the regulation of gene activity and expression which have been suggested by many researchers (Guo et al., 2004). More so, intra- and interspecific hybridisation is known to affect the expression and regulation of transcript between inbreed parents and F1-hybrids because of variation in their genetic background. Therefore, we wanted to understand the observed variation in gene regulation, expression and relative abundance in salt-treated *B. oleracea* genotypes could be due to allelic variation. The result has shown the presence of variants in many positions on the chromosomes and transcripts positions. Differences in nucleotide sequence observed at the same chromosomal positions where these genes are located could possibly be associated with the variation in gene expression and physiological response between DHSL150, wild S1 parents lines, and DH lines.

The results so far, revealed variation in the response of *B. oleracea* genotypes to salt stress but due to time constraint, an effort to map out and linked these specific phenotypes (QTLs) with different positions and segment of chromosomes could not be achieved. This can be achieved by using marker-assisted selection and other interesting analyses that could lead to a successful breeding break-through. This was frustrating and thus highlights the problem that can be surmountable. Meanwhile, further work can be suggested, which include interesting steps which might lead to the development of enhanced research genetic resources for *B. oleracea* C-genome that can be used in breeding for salt tolerant brassica vegetables in future. Indeed, such developments will benefit both the production of brassica vegetables and enhance quality of food production generally salvaging millions of lives at the same time improving the economic status of the world.

7.1 Future work

From the current study, it was made clear the existence of variation between the cultivated founder DHSL150, wild S1 parent and doubled haploid *B. oleracea* genotypes in response to salt stress. However, the degree of variation attributable to genetics or environment was not totally deciphered because plant materials collected in this study were from one source of replicate i.e., leaves. Therefore, other plant materials such as roots and shoots could also be looked at. By further analysis of these plant materials, a better understanding of the level variation and interactions at different stages between the plants and the environment could be obtained. Apart from direct phenotype measurements, further studies on other critical indicators such as chlorophyll content (chlorophyll a & b) (Rezaei et al., 2017), antioxidants and phenolic compounds could be measured. Ronald et al. (2005), suggested spectrophotometric and HPLC methods could be added because salt stress tolerance is a

complex phenomenon and there is crosstalk between many pathways. Furthermore, structural genes are involved in membrane stability and these could also be investigated by using qPCR to look at the changes in gene expression encoding these structural proteins required to maintain membrane stability under salt stress conditions. Different analytical approaches could also be applied to analyse the component of variance by using maximum likelihood analysis, which provides an unbiased estimation of different variation.

Many plant transporters that have been successfully linked to salt tolerance have been found through functional genetic analysis and were mapped out using QTL analysis. For example, QTLs for shoot K^+ content (SKC1), *Nax1* and *Nax2* genes mapped to locus involves in Na^+ removal from the xylem in the roots and QTLs for H^+ monovalent cation exchanger family (CHX) (Lin et al., 2004; James et al., 2006; Byrt et al., 2007). Therefore, identification of locus positions and possible alleles associated could be of significant where genetic map using molecular markers can be used to identify close proximity as it relates to quantitative traits. This could be the first critical step to fine map the candidate genes and genomic regions, which could be followed by marker-assisted selection (MAS). Furthermore, a backcross especially between the wild S1 and doubled haploid, can be applied and desired genotypes in resulting segregating populations could be used to map out superior genotypes with introgressed regions being broken down further. Such genetic resources can be used for gene identification. Alternatively, association studies could be carried out to identify those alleles that could be beneficial for the breeding program (Callow et al., 1997; Henry, 2001; Newbury, 2003).

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Appendices

Appendix I

Table S1: List of wild founder Parent lines (DFFS)

Brassica C genome DFFS founder lines

BCgDFS founder line	DFFS set No.	Species
BCgDFS_001	C04001	<i>alboglabra</i>
BCgDFS_002	C04002	<i>atlantica</i>
BCgDFS_003	C04003	<i>atlantica</i>
BCgDFS_004	C04005	<i>balearica</i>
BCgDFS_005	C04006	<i>bourgaei</i>
BCgDFS_006	C04007	<i>cretica</i>
BCgDFS_007	C04008	<i>cretica</i>
BCgDFS_008	C04009	<i>cretica</i>
BCgDFS_009	C04010	<i>cretica</i>
BCgDFS_010	C04011	<i>cretica</i>
BCgDFS_011	C04012	<i>cretica</i>
BCgDFS_012	C04013	<i>cretica</i>
BCgDFS_013	C04014	<i>cretica</i>
BCgDFS_014	C04015	<i>hilarionis</i>
BCgDFS_015	C04016	<i>hilarionis</i>
BCgDFS_016	C04017	<i>hilarionis</i>
BCgDFS_017	C04018	<i>incana</i>
BCgDFS_018	C04019	<i>incana</i>
BCgDFS_019	C04020	<i>incana</i>
BCgDFS_020	C04021	<i>incana</i>
BCgDFS_021	C04023	<i>incana</i>
BCgDFS_022	C04024	<i>insularis</i>
BCgDFS_023	C04027	<i>insularis</i>
BCgDFS_024	C04028	<i>macrocarpa</i>
BCgDFS_025	C04029	<i>macrocarpa</i>
BCgDFS_026	C04030	<i>macrocarpa</i>
BCgDFS_027	C04031	<i>macrocarpa</i>
BCgDFS_028	C04032	<i>macrocarpa</i>
BCgDFS_029	C04033	<i>macrocarpa</i>
BCgDFS_030	C04034	<i>macrocarpa</i>
BCgDFS_031	C04035	<i>macrocarpa</i>
BCgDFS_032	C04036	<i>macrocarpa</i>
BCgDFS_033	C04037	<i>macrocarpa</i>
BCgDFS_034	C04038	<i>macrocarpa</i>
BCgDFS_035	C04039	<i>macrocarpa</i>
BCgDFS_036	C04040	<i>macrocarpa</i>
BCgDFS_037	C04041	<i>macrocarpa</i>
BCgDFS_038	C04042	<i>macrocarpa</i>
BCgDFS_039	C04043	<i>macrocarpa</i>
BCgDFS_040	C04045	<i>montana</i>
BCgDFS_041	C04047	<i>oleracea</i>
BCgDFS_042	C04048	<i>oleracea</i>
BCgDFS_043	C04049	<i>oleracea</i>
BCgDFS_044	C04050	<i>oleracea</i>
BCgDFS_045	C04051	<i>oleracea</i>
BCgDFS_046	C04052	<i>oleracea</i>
BCgDFS_047	C04053	<i>oleracea</i>
BCgDFS_048	C04054	<i>oleracea</i>
BCgDFS_049	C04055	<i>oleracea</i>
BCgDFS_050	C04056	<i>oleracea</i>

Appendix I *Cont.*Table S1: *Cont.*

Brassica C genome DFFS founder lines

BCgDFS founder line	DFFS set No.	Species
BCgDFS_052	C04060	<i>oleracea</i>
BCgDFS_053	C04061	<i>oleracea</i>
BCgDFS_054	C04062	<i>oleracea</i>
BCgDFS_055	C04063	<i>oleracea</i>
BCgDFS_056	C04064	<i>oleracea</i>
BCgDFS_057	C04065	<i>oleracea</i>
BCgDFS_058	C04066	<i>oleracea</i>
BCgDFS_059	C04067	<i>oleracea</i>
BCgDFS_060	C04068	<i>oleracea</i>
BCgDFS_061	C04069	<i>oleracea</i>
BCgDFS_062	C04070	<i>montana</i>
BCgDFS_063	C04071	<i>montana</i>
BCgDFS_064	C04073	<i>rupestris</i>
BCgDFS_065	C04074	<i>rupestris</i>
BCgDFS_066	C04075	<i>rupestris</i>
BCgDFS_067	C04076	<i>rupestris</i>
BCgDFS_068	C04077	<i>incana</i>
BCgDFS_069	C04079	<i>incana</i>
BCgDFS_070	C04080	<i>incana</i>
BCgDFS_071	C04081	<i>incana</i>
BCgDFS_072	C04082	<i>incana</i>
BCgDFS_073	C04083	<i>villosa</i>
BCgDFS_074	C04084	<i>villosa</i>
BCgDFS_075	C04085	<i>villosa</i>
BCgDFS_076	C04086	<i>villosa</i>
BCgDFS_077	C04087	<i>villosa</i>
BCgDFS_078	C04088	<i>villosa</i>
BCgDFS_079	C04089	<i>villosa</i>
BCgDFS_080	C04090	<i>villosa</i>
BCgDFS_081	C04091	<i>villosa</i>
BCgDFS_082	C04092	<i>villosa</i>
BCgDFS_083	C04093	<i>villosa</i>
BCgDFS_084	C04094	<i>villosa</i>
BCgDFS_085	C04095	<i>villosa</i>
BCgDFS_086	C04096	<i>oleracea</i>
BCgDFS_087	C04097	<i>oleracea</i>
BCgDFS_088	C04098	<i>oleracea</i>

Appendix I *Cont.*

Table S2: List of wild S1 parent lines used in 2015 salt screening experiment.

Brassica C genome DFFS lines			
1	<i>Geno ID</i>	<i>Name</i>	Type
2	A12DHd	<i>B. oleracea</i>	DH
3	C04099	DHSL150 (C01001)	DH
4	C07007	<i>B. bourgaei</i>	S1
5	C07010	<i>B. cretica</i>	S1
6	C07011	<i>B. cretica</i>	S1
7	C07012	<i>B. cretica</i>	S1
8	C07014	<i>B. cretica</i>	S1
9	C07015	<i>B. cretica</i>	S1
10	C07017	<i>B. cretica</i>	S1
11	C07018	<i>B. cretica</i>	S1
12	C07019	<i>B. hilarionis</i>	S1
13	C07020	<i>B. hilarionis</i>	S1
14	C07023	<i>B. incana</i>	S1
15	C07024	<i>B. incana</i>	S1
16	C07026	<i>B. incana</i>	S1
17	C07030	<i>B. insularis</i>	S1
18	C07031	<i>B. macrocarpa</i>	S1
19	C07037	<i>B. macrocarpa</i>	S1
20	C07039	<i>B. macrocarpa</i>	S1
21	C07045	<i>B. macrocarpa</i>	S1
22	C07046	<i>B. macrocarpa</i>	S1
23	C07047	<i>B. macrocarpa</i>	S1
24	C07049	<i>B. macrocarpa</i>	S1
25	C07051	<i>B. montana</i>	S1
26	C07059	<i>B. oleracea</i>	S1
27	C07060	<i>B. oleracea</i>	S1
28	C07062	<i>B. oleracea</i>	S1
29	C07067	<i>B. oleracea</i>	S1
30	C07069	<i>B. oleracea</i>	S1
31	C07069A	<i>B. oleracea</i>	S1
32	C07071	<i>B. oleracea</i>	S1
33	C07073	<i>B. oleracea</i>	S1
34	C07075	<i>B. oleracea</i>	S1
35	C07077	<i>B. oleracea</i>	S1
36	C07078	<i>B. oleracea</i>	S1
37	C07079	<i>B. oleracea</i>	S1
38	C07079A	<i>B. oleracea</i>	S1
39	C07080	<i>B. montana</i>	S1
40	C07086	<i>B. rupestris</i>	S1
41	C07089	<i>B. rupestris</i>	S1
42	C07091	<i>B. incana</i> (listed	S1
43	C07093	<i>B. incana</i>	S1
44	C07094	<i>B. incana</i>	S1
45	C07095	<i>B. incana</i>	S1
46	C07103	<i>B. villosa bivonia</i>	S1
47	C07104	<i>B. villosa bivonia</i>	S1
48	C07113	<i>B. villosa tinei</i>	S1
49	C07123	<i>B. oleracea capita</i>	S1
50	Early Big	<i>B. oleracea</i>	DH
51	GDDH33	<i>B. oleracea</i>	DH
52	HRIGRU008	<i>B. oleracea</i>	
53	MarDH34	<i>B. oleracea</i>	DH

Appendix II

Table S2: Differences in Allelic variants at various chromosomes positions in parent lines; the cultivated rapid cycling DHSL150 and C07060.

Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060												
C1	38,213	T/T	C/C	C2	201,853	T/T	A/A	C2	47,958,970	A/A	G/G	C3	29,031,900	C/C	G/G	C4	24,881,517	G/G	T/T	C5	5,268,307	G/G	T/T	C5	41,376,151	G/G	C/C	C6	36,104,457	A/A	G/G	C7	42,708,449	T/T	G/G	C8	33,946,711	A/A	G/G	C9	20,272,618	G/G	A/A
C1	50,312	G/G	T/T	C2	211,730	G/G	A/A	C2	47,959,067	C/C	T/T	C3	29,031,925	G/G	C/C	C4	24,934,932	A/A	G/G	C5	5,289,694	T/T	C/C	C5	41,376,199	G/G	A/A	C6	36,104,476	T/T	A/A	C7	42,834,289	T/T	C/C	C8	33,946,718	T/T	C/C	C9	20,272,722	A/A	C/C
C1	315,837	A/A	G/G	C2	241,356	T/T	A/A	C2	47,982,206	A/A	G/G	C3	29,060,210	G/G	A/A	C4	25,342,584	T/T	G/G	C5	5,290,756	G/G	A/A	C5	41,382,029	T/T	C/C	C6	36,112,515	C/C	T/T	C7	42,876,601	T/T	C/C	C8	33,948,252	G/G	C/C	C9	20,272,735	C/C	T/T
C1	442,858	A/A	G/G	C2	255,727	C/C	T/T	C2	48,081,107	C/C	T/T	C3	29,105,463	C/C	T/T	C4	25,342,590	A/A	C/C	C5	5,345,566	A/A	G/G	C5	41,382,032	G/G	A/A	C6	36,148,763	A/A	G/G	C7	42,876,635	C/C	A/A	C8	33,948,337	C/C	T/T	C9	20,439,511	C/C	A/A
C1	442,876	C/C	T/T	C2	255,754	C/C	T/T	C2	48,130,962	C/C	T/T	C3	29,149,713	G/G	A/A	C4	25,352,624	A/A	G/G	C5	5,355,115	G/G	T/T	C5	41,390,781	A/A	C/C	C6	36,249,115	A/A	G/G	C7	42,877,087	A/A	T/T	C8	33,968,282	G/G	T/T	C9	20,542,127	G/G	C/C
C1	442,886	T/T	G/G	C2	395,705	A/A	T/T	C2	48,130,975	A/A	G/G	C3	29,149,730	G/G	C/C	C4	25,428,238	T/T	A/A	C5	5,367,105	G/G	A/A	C5	41,390,788	T/T	G/G	C6	36,341,716	C/C	G/G	C7	42,877,108	A/A	G/G	C8	33,968,319	A/A	G/G	C9	20,592,289	A/A	G/G
C1	467,057	G/G	A/A	C2	402,498	G/G	C/C	C2	48,195,834	A/A	G/G	C3	29,149,770	G/G	A/A	C4	25,466,901	A/A	C/C	C5	5,424,849	G/G	A/A	C5	41,401,004	A/A	G/G	C6	36,341,808	G/G	T/T	C7	42,887,875	C/C	A/A	C8	33,982,202	T/T	C/C	C9	20,616,341	A/A	G/G
C1	467,059	T/T	G/G	C2	402,527	T/T	C/C	C2	48,417,343	C/C	G/G	C3	29,173,179	C/C	T/T	C4	25,617,584	T/T	C/C	C5	5,439,089	T/T	A/A	C5	41,416,765	C/C	A/A	C6	36,341,812	C/C	T/T	C7	43,058,037	A/A	G/G	C8	34,019,166	A/A	T/T	C9	20,669,613	C/C	T/T
C1	647,111	C/C	T/T	C2	525,532	C/C	A/A	C2	48,639,626	G/G	A/A	C3	29,221,565	T/T	G/G	C4	25,729,507	A/A	T/T	C5	5,445,536	C/C	G/G	C5	41,753,273	G/G	A/A	C6	36,363,515	G/G	C/C	C7	43,058,058	T/T	C/C	C8	34,025,457	A/A	G/G	C9	20,705,584	C/C	A/A
C1	856,371	A/A	G/G	C2	527,017	G/G	A/A	C2	48,639,718	A/A	G/G	C3	29,342,249	C/C	T/T	C4	25,743,255	A/A	G/G	C5	5,445,560	C/C	T/T	C5	41,753,309	C/C	T/T	C6	36,367,455	A/A	G/G	C7	43,094,940	T/T	G/G	C8	34,025,712	C/C	T/T	C9	20,755,405	C/C	T/T
C1	1,019,546	A/A	C/C	C2	591,671	C/C	T/T	C2	48,639,815	A/A	C/C	C3	29,357,173	A/A	G/A	C4	25,747,613	G/G	T/T	C5	5,480,331	C/C	T/T	C5	41,774,258	T/T	C/C	C6	36,367,522	C/C	G/G	C7	43,094,963	C/C	T/T	C8	34,048,200	T/T	C/C	C9	20,755,466	C/C	G/G
C1	1,019,628	A/A	C/C	C2	591,689	T/T	C/C	C2	48,639,830	T/T	C/C	C3	29,357,209	T/T	A/A	C4	25,767,209	T/T	G/G	C5	5,480,351	T/T	C/C	C5	41,774,263	T/T	C/C	C6	36,367,544	T/T	G/G	C7	43,281,999	G/G	T/T	C8	34,058,082	C/C	G/G	C9	20,755,680	C/C	G/G
C1	1,105,498	A/A	G/G	C2	594,580	C/C	T/T	C2	48,648,060	T/T	G/G	C3	29,357,250	T/T	G/G	C4	25,767,212	T/T	C/C	C5	5,496,744	G/G	C/C	C5	41,850,817	G/G	T/T	C6	36,367,591	C/C	T/T	C7	43,282,052	A/A	T/T	C8	34,058,136	C/C	G/G	C9	20,783,254	T/T	C/C
C1	1,105,553	C/C	T/T	C2	675,339	A/A	T/T	C2	48,677,626	C/C	T/T	C3	29,518,511	C/C	A/A	C4	25,815,437	C/C	T/T	C5	5,496,801	A/A	G/G	C5	41,965,913	A/A	T/T	C6	36,367,614	C/A	C/C	C7	43,333,400	C/C	T/T	C8	34,072,393	G/G	A/A	C9	20,790,895	T/T	G/G
C1	1,138,452	A/A	G/G	C2	675,346	A/A	T/T	C2	48,907,302	A/A	T/T	C3	29,519,209	T/T	G/A	C4	25,815,473	C/C	T/T	C5	5,497,356	A/A	C/C	C5	41,965,913	A/A	T/T	C6	36,402,650	G/A	A/A	C7	43,451,522	C/C	T/T	C8	34,137,602	A/A	T/T	C9	20,888,559	G/G	C/C
C1	1,242,142	G/G	T/T	C2	694,385	T/T	G/G	C2	48,907,311	T/T	C/C	C3	29,519,210	T/T	C/C	C4	26,114,118	C/C	G/G	C5	5,497,384	G/G	T/T	C5	42,144,830	A/A	T/T	C6	36,402,693	A/A	G/G	C7	43,451,595	T/T	A/A	C8	34,137,638	A/A	C/C	C9	20,889,299	A/A	G/G
C1	1,242,469	A/A	G/G	C2	706,047	C/A	A/A	C2	48,907,328	G/G	T/T	C3	29,622,889	A/A	C/C	C4	26,171,845	T/T	A/A	C5	5,498,957	C/C	G/G	C5	42,144,863	T/T	A/A	C6	36,410,550	T/T	C/C	C7	43,451,672	G/G	C/C	C8	34,137,668	G/G	A/A	C9	21,003,826	A/A	C/C
C1	1,242,511	A/A	T/T	C2	734,016	A/A	G/G	C2	48,907,401	C/C	T/T	C3	29,625,267	C/A	A/A	C4	26,171,859	T/T	G/G	C5	5,638,796	G/G	T/T	C5	42,152,432	C/C	T/T	C6	36,572,172	C/C	T/T	C7	43,459,587	C/C	T/T	C8	34,262,371	T/T	C/C	C9	21,175,299	G/G	A/A
C1	1,336,381	T/T	G/G	C2	835,983	T/T	C/C	C2	48,907,415	C/C	T/T	C3	29,626,185	A/A	G/G	C4	26,171,863	T/T	G/G	C5	5,644,366	C/C	T/T	C5	42,204,939	C/C	T/T	C6	36,730,766	T/T	A/A	C7	43,461,649	C/C	T/T	C8	34,262,404	G/G	A/A	C9	21,263,044	T/T	C/C
C1	1,336,408	G/G	C/C	C2	943,534	G/G	T/T	C2	48,972,024	C/A	A/A	C3	29,626,274	C/A	G/G	C4	26,262,073	A/A	G/G	C5	5,662,949	G/G	A/A	C5	42,204,945	C/C	T/T	C6	36,750,902	G/A	A/A	C7	43,486,046	T/T	G/G	C8	34,262,488	A/A	G/G	C9	21,265,587	T/T	A/A
C1	1,336,412	A/A	T/T	C2	943,593	T/T	A/A	C2	49,218,286	C/C	T/T	C3	29,626,285	C/C	G/G	C4	26,262,091	T/T	G/G	C5	5,662,979	A/A	C/C	C5	42,205,046	T/T	A/A	C6	36,766,431	G/G	C/C	C7	43,489,851	G/G	C/C	C8	34,403,603	A/A	T/T	C9	21,506,741	G/G	A/A
C1	1,369,909	T/T	A/A	C2	943,595	A/A	T/T	C2	49,367,130	T/T	C/C	C3	29,626,286	A/A	G/G	C4	26,262,190	G/G	A/A	C5	5,718,767	C/C	A/A	C5	42,206,339	A/A	C/C	C6	36,970,480	C/C	G/G	C7	43,489,890	A/A	G/G	C8	34,410,244	A/A	T/T	C9	21,532,739	A/A	T/T
C1	1,377,974	C/C	T/T	C2	971,392	A/A	G/G	C2	49,377,684	C/C	A/A	C3	29,627,028	C/C	T/T	C4	26,262,897	G/G	A/A	C5	5,775,865	C/C	T/T	C5	42,206,462	A/A	C/C	C6	36,970,502	C/C	G/G	C7	43,489,902	G/G	A/A	C8	34,451,344	T/T	C/C	C9	21,532,823	T/T	C/C
C1	1,377,993	A/A	G/G	C2	997,767	C/C	T/T	C2	49,427,256	G/G	C/C	C3	29,627,356	T/T	C/C	C4	26,262,906	T/T	C/C	C5	5,775,881	T/T	C/C	C5	42,206,490	A/A	T/T	C6	36,970,504	A/A	G/G	C7	43,532,861	C/C	A/A	C8	34,451,362	T/T	A/A	C9	21,537,127	T/T	C/C
C1	1,377,998	T/T	C/C	C2	1,033,429	A/A	C/C	C2	49,588,385	G/G	T/T	C3	29,627,530	G/A	C/C	C4	26,263,514	G/C	C/C	C5	5,782,246	A/A	C/C	C5	42,209,187	C/C	T/T	C6	36,972,592	T/T	A/A	C7	43,533,047	G/G	A/A	C8	34,451,399	T/T	G/G	C9	21,551,284	A/A	C/C
C1	1,378,051	T/T	G/G	C2	1,104,244	C/C	G/G	C2	49,588,485	G/G	C/C	C3	29,627,597	G/G	T/T	C4	26,263,553	T/T	C/C	C5	5,782,278	C/C	T/T	C5	42,212,960	G/G	A/A	C6	36,983,143	T/T	C/C	C7	43,624,963	A/A	C/C	C8	34,452,644	C/C	T/T	C9	21,551,340	G/G	A/A
C1	1,378,172	A/A	C/C	C2	1,104,294	A/A	G/G	C2	49,698,532	C/C	T/T	C3	30,128,971	C/C	T/T	C4	26,263,568	G/G	T/T	C5	5,844,716	A/A	T/T	C5	42,221,926	T/T	C/C	C6	36,983,185	G/G	A/A	C7	43,625,049	T/T	A/A	C8	34,452,646	T/T	C/C	C9	21,947,262	T/T	A/A
C1	1,425,040	C/C	A/A	C2	1,106,862	G/A	A/A	C2	49,713,183	C/C	T/T	C3	30,129,012	C/C	A/A	C4	26,263,879	G/G	A/A	C5	5,844,748	T/T	C/C	C5	42,434,087	C/C	T/T	C6	36,983,272	T/T	C/C	C7	43,625,109	A/A	G/G	C8	34,585,379	G/G	A/A	C9	22,166,843	A/A	G/G
C1	1,425,746	A/A	C/C	C2	1,128,434	T/T	C/C	C2	49,713,303	C/C	T/T	C3	30,129,072	C/C	T/T	C4	26,309,268	C/C	T/T	C5	5,848,684	G/G	C/C	C5	42,620,136	C/A	A/A	C6	36,984,235	T/T	G/G	C7	43,625,642	T/T	C/C	C8	34,635,140	C/C	T/T	C9	22,166,857	A/A	C/C
C1	1,443,929	C/C	T/T	C2	1,188,782	C/A	A/A	C2	49,794,994	G/G	C/C	C3	30,129,089	C/C	T/T	C4	26,352,381	T/T	A/A	C5	5,848,726	C/A	A/A	C5	42,620,177	A/A	G/G	C6	36,984,246	G/G	A/A	C7	43,625,775	C/C	G/G	C8	34,641,302	C/C	T/T	C9	22,211,641	C/C	T/T
C1	1,450,645	C/C	G/G	C2	1,188,883	G/G	A/A	C2	50,052,367	T/T	A/A	C3	30,220,676	G/G	A/A	C4	26,353,929	A/A	C/C	C5	5,848,735	A/A	G/G	C5	42,650,547	T/T	C/C	C6	36,984,379	A/A	G/G	C7	43,625,850	C/C	T/T	C8	34,641,306	A/A	T/T	C9	22,271,663	A/A	T/T
C1	1,451,104	G/G	A/A	C2	1,264,601	T/T	G/G	C2	50,052,409	C/C	T/T	C3	30,334,014	T/T	G/G	C4	26,367,368	T/T	C/C	C5	5,874,007	T/T	G/G	C5	42,694,205	A/A	G/G	C6	36,984,394	T/T	G/G	C7	43,625,853	T/T	C/C	C8	34,692,826	G/G	T/T	C9	22,325,428	A/A	G/G
C1	1,687,775	C/C	T/T	C2	1,264,729	A/A	T/T	C2	50,136,949	A/A	G/G	C3	30,334,104	C/C	G/G	C4	26,520,204	A/A	C/C	C5	5,968,823	C/C	T/T	C5	42,694,235	C/C	T/T	C6	36,991,487	A/A	G/G	C7	43,760,423	A/A	G/G	C8	34,768,869	C/C	T/T	C9	22,575,192		

Appendix II *Cont.***Table S2: *Cont.* : Differences in Allelic variants at various chromosomes positions in parent lines; the cultivated rapid cycling DHSL150 and C07060.**

Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060												
C1	2,008,839	C/C	T/T	C2	1,570,143	C/C	T/T	C2	51,558,684	A/A	C/C	C3	31,991,402	G/G	A/A	C4	27,286,680	A/A	G/G	C5	6,788,221	T/T	G/G	C5	43,418,157	G/G	T/T	C6	37,289,161	T/T	C/C	C7	44,113,394	C/C	A/A	C8	35,335,414	A/A	C/C	C9	28,007,732	G/G	A/A
C1	2,025,250	G/G	A/A	C2	1,929,688	T/T	C/C	C2	51,782,672	G/G	A/A	C3	32,021,781	G/G	A/A	C4	27,291,604	A/A	T/T	C5	6,819,385	C/C	T/T	C5	43,418,185	G/G	A/A	C6	37,289,290	T/T	G/G	C7	44,119,246	C/C	T/T	C8	35,443,100	C/C	G/G	C9	28,007,761	G/G	A/A
C1	2,067,679	G/G	T/T	C2	1,929,703	G/G	C/C	C2	51,885,119	T/T	C/C	C3	32,021,854	T/T	C/C	C4	27,318,425	C/A	A/A	C5	6,986,306	T/T	A/A	C5	43,438,289	T/T	C/C	C6	37,293,244	A/A	C/C	C7	44,199,407	C/C	T/T	C8	35,561,433	T/T	C/C	C9	28,207,220	C/C	A/A
C1	2,067,719	C/C	G/A	C2	1,929,720	G/G	A/A	C2	51,885,128	C/A	T/T	C3	32,120,175	A/A	G/G	C4	27,349,693	C/C	G/G	C5	6,988,118	G/G	A/A	C5	43,438,852	C/C	T/T	C6	37,293,268	G/G	T/T	C7	44,274,649	C/C	A/A	C8	35,571,055	G/G	T/T	C9	28,207,293	A/A	T/T
C1	2,071,193	G/G	A/A	C2	1,931,177	C/G	G/G	C2	51,885,212	G/G	A/A	C3	32,209,934	A/A	T/T	C4	27,445,670	G/A	A/A	C5	6,988,128	G/G	A/A	C6	37,293,682	A/A	G/G	C7	44,366,915	G/G	C/C	C8	35,571,071	C/C	A/A	C9	28,335,493	T/T	C/C				
C1	2,071,195	G/G	C/C	C2	1,933,920	G/G	T/T	C2	51,885,228	C/G	G/G	C3	32,226,270	T/T	G/G	C4	27,495,764	A/A	G/G	C5	7,062,469	C/C	G/G	C5	43,446,258	C/C	G/G	C6	37,446,539	A/A	G/G	C7	44,498,653	T/T	C/C	C8	35,598,844	T/T	A/A	C9	28,378,245	A/A	G/G
C1	2,095,969	G/G	A/A	C2	1,974,601	G/A	A/A	C2	52,046,753	A/A	C/C	C3	32,249,119	C/A	T/T	C4	27,495,783	C/C	C/C	C5	7,068,241	A/A	C/C	C5	43,446,269	G/G	A/A	C6	37,570,287	A/A	C/C	C7	44,498,697	T/T	C/C	C8	35,699,752	A/A	T/T	C9	28,594,730	C/C	T/T
C1	2,096,077	A/A	T/T	C2	1,974,614	A/A	C/C	C2	52,046,782	G/G	C/C	C3	32,295,401	C/A	A/A	C4	27,495,797	A/A	C/C	C5	7,069,487	A/A	T/T	C5	43,472,729	C/C	T/T	C6	37,570,479	T/T	C/C	C7	44,498,760	G/G	T/T	C8	35,699,762	G/G	A/A	C9	28,594,733	A/A	G/G
C1	2,096,086	A/A	G/G	C2	1,974,746	A/A	C/C	C2	52,056,838	A/A	C/C	C3	32,333,586	C/A	T/T	C4	27,509,184	C/C	T/T	C5	7,113,547	T/T	C/C	C5	43,498,082	C/C	T/T	C6	37,680,483	C/C	T/T	C7	44,498,793	C/C	T/T	C8	35,736,389	G/G	A/A	C9	28,594,823	G/G	A/A
C1	2,101,416	G/G	A/A	C2	1,991,113	C/A	T/T	C2	52,110,476	T/T	C/C	C3	32,333,598	G/A	A/A	C4	27,694,572	C/C	G/G	C5	7,175,484	T/T	C/C	C5	43,563,458	A/A	G/G	C6	37,680,491	C/C	T/T	C7	44,558,338	A/A	T/T	C8	35,780,462	A/A	T/T	C9	28,594,847	A/A	C/C
C1	2,165,570	G/G	A/A	C2	2,154,992	A/A	G/G	C2	52,271,094	T/T	C/C	C3	32,379,347	C/C	T/T	C4	27,725,053	A/A	G/G	C5	7,181,790	T/T	C/C	C5	43,563,497	C/C	T/T	C6	37,680,516	C/C	A/A	C7	44,647,788	G/G	A/A	C8	35,780,485	A/A	G/G	C9	28,662,383	A/A	G/G
C1	2,165,588	C/C	A/A	C2	2,160,128	C/A	T/T	C2	52,303,209	A/A	C/C	C3	32,379,358	T/T	C/C	C4	27,868,868	T/T	G/G	C5	7,181,885	A/A	T/T	C5	43,564,822	A/A	C/C	C6	37,717,850	C/A	A/A	C7	44,647,797	T/T	G/G	C8	35,797,152	G/G	T/T	C9	28,730,082	G/G	A/A
C1	2,165,597	T/T	C/C	C2	2,169,934	C/A	T/T	C2	52,329,510	C/C	T/T	C3	32,379,410	A/A	G/G	C4	27,866,907	C/C	T/T	C5	7,197,113	A/A	G/G	C5	43,564,878	G/A	A/A	C6	37,793,344	G/A	A/A	C7	44,647,821	C/C	T/T	C8	35,811,711	A/A	T/T	C9	28,830,692	T/T	C/C
C1	2,170,819	T/T	C/C	C2	2,191,264	C/C	G/G	C2	52,333,656	C/C	G/G	C3	32,374,034	A/A	G/G	C4	27,869,006	C/C	T/T	C5	7,201,354	G/G	C/C	C5	43,565,778	G/G	A/A	C6	37,830,079	A/A	G/G	C7	44,647,824	T/T	C/C	C8	35,811,902	G/G	A/A	C9	28,752,787	G/G	A/A
C1	2,170,837	T/T	A/A	C2	2,191,267	T/T	C/C	C2	52,333,676	G/A	A/A	C3	32,583,943	T/T	G/A	C4	27,894,059	G/C	C/C	C5	7,302,901	G/C	A/A	C5	43,574,475	C/C	A/A	C6	37,830,088	G/C	T/T	C7	44,648,801	C/C	T/T	C8	35,818,400	T/T	A/A	C9	28,759,046	G/G	A/A
C1	2,170,870	A/A	G/A	C2	2,191,291	G/A	A/A	C2	52,457,961	G/C	C/C	C3	32,781,382	C/C	A/A	C4	28,111,519	C/C	T/T	C5	7,382,702	C/C	T/T	C5	43,586,946	T/T	A/A	C6	37,927,157	A/A	T/A	C7	44,679,341	G/A	A/A	C8	35,821,962	G/C	C/C	C9	28,944,637	G/G	A/A
C1	2,403,649	C/C	G/G	C2	2,193,128	G/G	T/T	C2	52,621,453	T/T	C/C	C3	33,775,207	T/T	A/A	C4	28,123,763	T/T	C/C	C5	7,446,769	T/T	C/C	C5	43,586,952	A/A	G/G	C6	37,955,134	T/T	C/C	C7	44,748,912	C/C	G/G	C8	35,856,349	T/T	C/C	C9	29,261,427	G/G	C/C
C1	2,403,680	T/T	C/C	C2	2,217,502	G/C	C/C	C2	52,680,644	A/A	T/T	C3	33,865,412	T/T	A/A	C4	28,314,049	C/C	T/T	C5	7,446,876	A/A	G/G	C5	43,586,961	A/A	G/G	C6	37,955,173	A/A	T/T	C7	44,773,436	A/A	G/G	C8	35,905,876	A/A	T/T	C9	29,600,784	C/C	T/T
C1	2,404,325	A/A	G/C	C2	2,244,075	G/G	T/T	C2	52,680,661	G/A	A/A	C3	33,865,441	C/C	T/T	C4	28,315,645	T/T	C/C	C5	7,460,966	G/G	A/A	C5	43,587,077	G/G	T/T	C6	37,955,349	T/T	G/T	C7	44,773,474	T/T	A/A	C8	35,946,606	C/C	G/G	C9	29,997,503	A/A	C/A
C1	2,405,411	A/A	G/C	C2	2,252,296	G/G	T/T	C3	349,244	A/A	T/T	C3	34,215,544	T/T	C/C	C4	28,315,695	G/C	C/C	C5	7,495,973	G/C	C/C	C5	43,587,120	A/A	G/G	C6	37,955,378	C/C	T/T	C7	44,799,786	G/C	C/C	C8	36,018,097	G/G	C/C	C9	30,144,348	A/A	G/G
C1	2,405,495	T/T	G/G	C2	2,266,939	C/C	T/T	C3	349,246	C/C	G/G	C3	34,256,115	C/C	T/T	C4	28,322,970	C/C	T/T	C5	7,530,646	C/C	A/A	C5	43,588,834	T/T	A/A	C6	37,975,142	C/C	T/T	C7	44,841,289	T/T	A/A	C8	36,105,111	T/T	C/C	C9	30,561,943	T/T	C/C
C1	2,489,091	T/T	G/G	C2	2,492,400	A/A	G/G	C3	349,339	T/T	A/A	C3	34,309,368	G/G	A/A	C4	28,460,048	C/C	T/T	C5	7,531,129	T/T	C/C	C5	43,588,909	C/A	A/A	C6	38,040,024	T/T	C/C	C7	44,889,309	A/A	G/G	C8	36,179,019	G/G	C/C	C9	30,593,182	C/C	G/G
C1	2,515,005	T/T	C/C	C2	2,492,875	G/G	A/A	C3	424,601	C/C	T/T	C3	34,309,771	T/T	C/C	C4	28,460,103	T/T	C/C	C5	7,531,150	C/C	G/G	C5	43,594,296	G/G	A/A	C6	38,179,045	A/A	C/A	C7	44,889,365	G/G	A/A	C8	36,256,128	C/C	T/T	C9	30,788,883	C/C	A/A
C1	2,515,077	G/G	A/A	C2	2,493,343	T/T	C/C	C3	555,897	A/A	T/T	C3	34,344,068	C/C	T/T	C5	7,594,328	C/C	T/T	C5	7,594,328	C/C	T/T	C5	43,608,978	C/C	A/A	C6	38,242,903	T/T	A/A	C7	44,940,969	G/C	C/C	C8	36,292,930	C/C	T/T	C9	30,870,370	T/T	C/C
C1	2,515,090	C/C	T/T	C2	2,503,194	A/A	T/T	C3	555,911	G/A	A/A	C3	34,450,536	G/C	C/C	C4	29,073,729	C/C	T/T	C5	7,594,312	C/C	T/T	C5	43,609,018	A/A	G/G	C6	38,298,703	T/T	C/C	C7	45,150,686	G/G	T/T	C8	36,429,099	T/T	C/C	C9	32,613,367	G/G	C/C
C1	2,586,742	T/T	C/C	C2	2,513,679	T/T	A/A	C3	555,994	C/C	T/T	C3	34,450,549	G/C	C/C	C4	29,074,559	T/T	C/C	C5	7,594,332	A/A	G/G	C5	43,618,767	A/A	G/G	C6	38,369,497	C/C	T/T	C7	45,163,516	C/C	G/G	C8	36,429,129	G/G	A/A	C9	32,949,220	A/A	C/C
C1	2,606,590	G/C	A/A	C2	2,528,990	G/G	A/A	C3	574,109	T/T	C/C	C3	34,450,562	C/C	T/T	C4	29,814,504	C/C	G/G	C5	7,595,252	T/T	A/A	C5	43,619,160	A/A	G/G	C6	38,369,543	C/C	T/T	C7	45,169,204	C/C	T/T	C8	36,681,006	A/A	G/G	C9	33,188,467	G/G	C/C
C1	2,635,717	G/G	A/A	C2	2,536,936	T/T	C/C	C3	576,418	C/G	G/G	C3	34,461,061	A/A	T/T	C4	29,086,814	C/A	A/A	C5	7,595,292	G/A	A/A	C5	43,619,283	C/C	T/T	C6	38,376,942	A/A	G/G	C7	45,247,098	C/C	T/T	C8	36,687,700	G/C	C/C	C9	33,208,851	A/A	C/C
C1	2,740,440	A/A	G/A	C2	2,616,893	C/C	T/T	C3	592,497	G/A	A/A	C3	34,479,741	G/A	A/A	C4	29,382,037	A/A	G/G	C5	7,595,313	G/G	A/A	C5	43,619,312	A/A	C/C	C6	38,377,067	T/T	G/G	C7	45,247,116	G/G	A/A	C8	36,702,481	T/T	C/C	C9	33,253,850	C/A	A/A
C1	2,768,880	T/T	C/C	C2	2,616,920	C/C	A/A	C3	618,702	C/C	T/T	C3	34,625,236	T/T	C/C	C4	29,382,808	C/C	A/A	C5	7,595,328	G/G	T/T	C5	43,767,559	T/T	C/C	C6	38,377,100	T/T	G/G	C7	45,247,158	C/C	T/T	C8	36,702,503	A/A	G/G	C9	33,360,128	G/G	T/T
C1	2,810,257	A/A	G/G	C2	2,630,222	A/A	G/G	C3	619,249	T/T	C/C	C3	34,747,783	G/G	A/A	C4	29,602,230	G/C	C/C	C5	7,669,660	C/C	T/T	C5	43,810,418	C/C	T/T	C6	38,377,109	G/G	T/T	C7	45,247,212	A/A	C/C	C8	36,775,807	G/G	A/A	C9	33,360,231	C/C	T/T
C1	2,810,286	A/A	G/G	C2	2,633,305	T/T	C/C	C3	638,764	T/T	G/G	C3	34,747,822	G/C	C/C	C4	29,811,345	C/C	A/A	C5	7,669,726	G/G	A/A	C5	43,810,424	T/T	C/C	C6	38,471,239	A/A	G/G	C7	45,314,993	C/A	A/A	C8	36,779,730	C/C	T/T	C9	33,648,825	C/C	T/T
C1	2,825,672	C/C	T/T	C2	2,667,008	A/A	G/G	C3	638,785	C/C	T/T	C3	34,747,844	C/C	T/T	C4	29,814,424	G/A	A/A	C5	7,689,208	T/T	A/A	C5	43,872,478	C/C	T/T	C6	38,493,446	T/T	C/C	C7	45,315,069	C/C	T/T	C8	36,779,774	T/T	C/C	C9	34,10		

Appendices

Appendix II Cont.

Table S2: Cont. : Differences in Allelic variants at various chromosomes positions in parent lines; the cultivated rapid cycling DHSL150 and C07060.

Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060												
C1	2,963,283	A/A	T/T	C2	3,008,042	T/T	C/C	C3	1,004,594	G/G	A/A	C3	36,344,421	T/T	C/C	C4	31,432,394	G/G	A/A	C5	8,039,084	A/A	G/G	C5	44,562,968	T/T	C/C	C6	38,929,073	G/G	T/T	C7	45,771,370	T/T	C/C	C8	37,253,053	A/A	G/G	C9	36,619,935	G/G	A/A
C1	2,988,196	T/T	A/A	C2	3,025,537	G/G	T/T	C3	1,004,722	C/C	G/G	C3	36,349,634	T/T	C/C	C4	31,572,201	T/T	G/G	C5	8,039,120	C/C	A/A	C5	44,562,995	T/T	C/C	C6	38,929,229	T/T	G/G	C7	45,771,434	C/C	G/G	C8	37,264,033	T/T	C/C	C9	36,620,044	C/C	T/T
C1	3,020,410	G/G	A/A	C2	3,025,695	C/C	A/A	C3	1,004,819	G/G	T/T	C3	36,365,707	G/G	C/C	C4	31,572,283	A/A	C/C	C5	8,042,937	C/C	T/T	C5	44,595,720	T/T	C/C	C6	38,929,230	T/T	G/G	C7	45,772,542	G/G	A/A	C8	37,264,070	C/C	T/T	C9	36,646,233	C/C	T/T
C1	3,020,704	G/G	A/A	C2	3,066,987	T/T	C/C	C3	1,037,668	C/C	A/A	C3	36,365,809	C/C	A/A	C4	31,684,025	G/G	T/T	C5	8,043,021	G/G	A/A	C5	44,595,721	G/G	A/A	C6	38,961,195	G/G	C/C	C7	45,788,775	A/A	T/T	C8	37,322,934	T/T	A/A	C9	36,646,295	A/A	C/C
C1	3,050,403	A/A	T/T	C2	3,066,993	T/T	C/C	C3	1,037,711	G/G	A/A	C3	36,365,830	G/G	T/T	C4	31,687,920	T/T	G/G	C5	8,054,935	A/A	C/C	C5	44,595,768	T/T	G/G	C6	38,970,934	C/C	T/T	C7	45,869,400	A/A	G/G	C8	37,440,274	T/T	A/A	C9	36,976,345	A/A	C/C
C1	3,085,264	G/G	T/T	C2	3,114,760	A/A	T/T	C3	1,040,821	A/A	G/G	C3	36,369,710	A/A	C/C	C4	31,714,545	A/A	C/C	C5	8,055,064	C/C	A/A	C5	44,628,081	A/A	G/G	C6	38,970,937	T/T	C/C	C7	45,902,609	T/T	C/C	C8	37,440,364	A/A	T/T	C9	37,068,043	A/A	G/G
C1	3,085,285	A/A	G/G	C2	3,138,452	C/C	G/G	C3	1,040,822	T/T	A/A	C3	36,369,711	T/T	G/G	C4	31,823,278	T/T	G/G	C5	8,055,190	A/A	G/G	C5	44,723,672	G/G	T/T	C6	39,111,099	A/A	C/C	C7	45,902,734	T/T	C/C	C8	37,466,502	G/G	C/C	C9	37,133,293	A/A	G/G
C1	3,136,275	G/G	A/A	C2	3,152,627	A/A	T/T	C3	1,045,856	A/A	G/G	C3	36,369,744	C/C	A/A	C4	31,823,288	T/T	G/G	C5	8,080,343	G/G	C/C	C5	44,832,965	A/A	G/G	C6	39,179,264	T/T	C/T	C7	45,902,786	G/G	A/A	C8	37,496,185	C/C	T/T	C9	37,269,758	A/A	T/T
C1	3,147,281	A/A	C/C	C2	3,152,858	T/T	G/G	C3	1,045,896	T/T	C/C	C3	36,374,770	T/T	A/A	C4	31,909,836	A/A	G/G	C5	8,190,120	A/A	G/G	C5	44,833,080	A/A	G/G	C6	39,179,268	A/A	T/T	C7	45,911,331	C/C	T/T	C8	37,496,198	C/C	T/T	C9	37,269,825	G/G	C/C
C1	3,216,979	T/T	G/G	C2	3,161,907	C/C	A/A	C3	1,106,834	G/G	A/A	C3	36,374,844	G/G	A/A	C4	32,006,458	C/C	T/T	C5	8,190,197	C/C	T/T	C5	44,833,095	G/G	C/C	C6	39,179,964	T/T	C/C	C7	45,922,757	A/A	G/G	C8	37,496,268	G/G	A/A	C9	37,271,835	C/C	T/T
C1	3,371,471	A/A	G/G	C2	3,182,010	A/A	T/T	C3	1,106,923	T/T	G/G	C3	36,374,931	C/C	T/T	C4	32,603,913	C/C	T/T	C5	8,190,212	G/G	A/A	C5	44,846,179	C/C	G/G	C6	39,307,933	A/A	C/C	C7	46,005,669	A/A	G/G	C8	37,503,113	A/A	G/G	C9	37,345,382	T/T	C/C
C1	3,374,485	T/T	C/C	C2	3,182,025	A/A	G/G	C3	1,112,767	A/A	C/C	C3	36,374,940	T/T	C/C	C4	32,618,358	G/G	C/C	C5	8,276,629	G/G	C/C	C5	44,868,355	T/T	G/G	C6	39,308,071	T/T	C/C	C7	46,019,997	G/G	A/A	C8	37,503,630	A/A	G/G	C9	37,434,781	C/A	A/A
C1	3,408,660	G/G	A/A	C2	3,182,082	G/G	A/A	C3	1,116,402	C/A	A/A	C3	36,395,077	C/C	G/G	C4	32,641,835	T/T	G/G	C5	8,370,086	T/T	C/C	C5	44,869,642	T/T	C/C	C6	39,317,339	G/G	C/C	C7	46,020,002	C/C	T/T	C8	37,504,115	G/G	A/A	C9	37,434,794	C/C	T/T
C1	3,409,694	T/T	C/C	C2	3,368,330	A/A	G/G	C3	1,116,441	C/C	T/T	C3	36,448,981	A/A	G/G	C4	32,867,332	C/C	T/T	C5	8,426,990	T/T	C/C	C5	44,928,813	C/C	T/T	C6	39,350,164	G/G	A/A	C7	46,020,022	C/C	G/G	C8	37,519,184	T/T	C/C	C9	37,434,806	T/T	C/C
C1	3,515,326	A/A	G/G	C2	3,368,336	G/G	A/A	C3	1,116,444	A/A	T/T	C3	36,514,510	G/G	A/A	C4	32,867,489	A/A	G/G	C5	8,457,889	C/C	T/T	C5	44,928,822	A/A	T/T	C6	39,407,586	G/G	A/A	C7	46,022,413	G/G	T/T	C8	37,519,244	T/T	C/C	C9	37,443,531	A/A	G/G
C1	3,630,797	T/T	G/G	C2	3,350,194	A/A	G/G	C3	1,116,528	G/G	T/T	C3	36,514,593	G/G	T/T	C4	32,920,992	T/T	G/G	C5	8,458,076	C/C	G/G	C5	44,963,587	C/C	T/T	C6	39,412,522	C/C	A/A	C7	46,022,542	G/G	A/A	C8	37,566,732	A/A	G/G	C9	37,479,471	A/A	G/G
C1	3,630,949	G/G	C/C	C2	3,602,744	G/G	A/A	C3	1,116,690	A/A	T/T	C3	36,570,496	A/A	G/G	C4	32,955,076	G/G	A/A	C5	8,472,947	T/T	C/C	C5	44,963,631	A/A	G/G	C6	39,420,626	G/G	A/A	C7	46,049,939	G/G	A/A	C8	37,566,758	A/A	G/G	C9	37,479,555	G/G	A/A
C1	3,630,982	A/A	C/C	C2	3,602,786	A/A	C/C	C3	1,128,764	C/C	T/T	C3	36,600,570	C/C	T/T	C4	32,955,114	G/G	A/A	C5	8,492,967	A/A	C/C	C5	45,015,144	C/A	A/A	C6	39,420,629	A/A	G/G	C7	46,050,086	A/A	G/G	C8	37,566,771	A/A	G/G	C9	37,577,429	A/A	G/G
C1	3,630,994	G/G	C/C	C2	3,711,838	T/T	A/A	C3	1,224,759	G/G	A/A	C3	36,600,591	C/C	T/T	C4	33,324,051	C/C	T/T	C5	8,646,257	T/T	A/A	C5	45,015,173	C/C	T/T	C6	39,427,433	T/T	C/C	C7	46,106,063	C/C	T/T	C8	37,566,809	C/C	G/G	C9	37,577,431	T/T	C/C
C1	3,631,006	G/G	C/C	C2	3,713,850	C/A	A/A	C3	1,271,839	C/C	T/T	C3	36,600,624	A/A	G/G	C4	33,759,452	C/A	A/A	C5	8,689,030	G/G	T/T	C5	45,110,998	T/T	C/C	C6	39,450,543	T/T	C/C	C7	46,108,476	C/C	T/T	C8	37,649,187	T/T	G/G	C9	37,708,409	G/G	T/T
C1	3,632,575	A/A	G/G	C2	3,740,776	G/G	C/C	C3	1,292,528	G/G	A/A	C3	36,621,067	A/A	G/G	C4	33,843,314	T/T	C/C	C5	8,689,045	A/A	C/C	C5	45,307,412	C/C	T/T	C6	39,472,945	G/G	T/T	C7	46,112,480	T/T	A/A	C8	37,654,538	T/T	G/G	C9	37,708,556	C/C	T/T
C1	3,640,305	T/T	C/C	C2	3,740,811	A/A	T/T	C3	1,339,226	T/T	C/C	C3	36,621,070	G/A	A/A	C4	33,843,339	G/G	A/A	C5	8,689,069	C/C	T/T	C5	45,307,437	C/C	T/T	C6	39,594,005	C/C	T/T	C7	46,155,616	T/T	C/C	C8	37,687,660	G/G	A/A	C9	37,963,560	T/T	A/A
C1	3,678,779	C/C	G/G	C2	3,742,022	C/C	T/T	C3	1,339,289	C/C	T/T	C3	36,629,262	T/T	C/C	C4	33,992,285	C/C	T/T	C5	8,689,075	T/T	C/C	C5	45,342,879	T/T	A/A	C6	39,809,118	T/T	G/G	C7	46,176,412	T/T	C/C	C8	37,687,680	T/T	C/A	C9	38,007,893	G/G	C/C
C1	3,678,855	G/G	A/A	C2	3,742,122	C/C	T/T	C3	1,339,906	T/T	C/C	C3	36,629,103	C/C	A/A	C4	33,844,259	T/T	C/C	C5	8,767,070	A/A	C/C	C5	45,356,292	A/A	G/G	C7	152,990	T/T	A/A	C7	46,287,759	A/A	G/G	C8	37,687,686	T/T	C/C	C9	38,012,201	A/A	C/C
C1	3,678,896	C/C	G/G	C2	3,742,149	C/A	A/A	C3	1,339,918	C/C	T/T	C3	36,629,127	C/C	T/T	C4	33,965,208	C/C	G/G	C5	8,915,124	G/G	T/T	C5	45,356,325	T/T	C/C	C7	445,998	C/C	G/G	C7	46,296,042	G/G	A/A	C8	37,689,626	G/G	C/C	C9	38,012,206	C/C	T/T
C1	3,685,691	A/A	G/G	C2	3,839,305	G/G	A/A	C3	1,339,979	A/A	G/G	C3	36,629,262	T/T	C/C	C4	33,992,285	C/C	T/T	C5	9,327,284	A/A	C/C	C5	45,489,496	C/C	A/A	C7	922,130	A/A	C/C	C7	46,296,166	G/G	A/A	C8	37,719,235	A/A	T/T	C9	38,012,288	C/C	T/T
C1	3,685,742	G/G	C/C	C2	3,990,650	C/A	A/A	C3	1,339,980	A/A	G/G	C3	36,736,368	C/C	A/A	C4	34,035,569	G/G	T/T	C5	9,421,019	A/A	C/C	C5	45,494,689	G/G	A/A	C7	977,952	A/A	G/G	C7	46,296,176	T/T	C/C	C8	37,719,247	C/C	G/A	C9	38,110,773	T/T	C/C
C1	3,708,787	A/A	G/G	C2	4,034,504	C/C	A/A	C3	1,341,941	G/G	C/C	C3	36,761,520	A/A	C/C	C4	34,112,474	T/T	G/G	C5	9,588,385	T/T	G/G	C5	45,545,136	A/A	G/G	C7	977,976	C/C	T/T	C7	46,298,925	T/T	G/G	C8	37,764,351	G/G	A/A	C9	38,110,775	T/T	A/A
C1	3,731,201	C/C	T/T	C2	4,075,843	A/A	G/G	C3	1,341,953	T/T	C/C	C3	36,761,529	G/G	A/A	C4	34,241,598	C/C	A/A	C5	9,588,393	C/C	A/A	C5	45,611,727	C/C	T/T	C7	1,092,216	C/C	T/T	C7	46,301,123	T/T	A/A	C8	37,797,695	G/G	C/C	C9	38,110,917	T/T	C/C
C1	3,769,610	T/T	A/A	C2	4,107,378	A/A	G/G	C3	1,341,968	A/A	G/G	C3	36,762,133	G/G	A/A	C4	34,241,659	A/A	G/G	C5	9,588,406	T/T	C/C	C5	45,611,729	G/G	A/A	C7	1,208,722	T/T	C/C	C7	46,308,739	G/G	A/A	C8	37,797,714	G/G	A/A	C9	38,128,143	C/C	T/T
C1	3,771,944	G/G	A/A	C2	4,110,312	G/G	A/A	C3	1,378,633	A/A	G/G	C3	36,765,984	T/T	A/A	C4	34,241,992	G/G	A/A	C5	9,595,661	C/C	T/T	C5	45,687,094	T/T	A/A	C7	1,248,275	C/C	T/T	C7	46,308,917	C/C	A/A	C8	37,797,746	A/A	C/C	C9	38,153,542	A/A	G/G
C1	4,019,528	A/A	G/G	C2	4,110,318	A/A	T/T	C3	1,476,791	C/C	T/T	C3	36,786,929	A/A	G/G	C4	34,596,615	G/G	C/C	C5	9,625,083	G/G	T/T	C5	45,801,810	A/A	G/G	C7	1,248,284	T/T	C/C	C7	46,332,003	G/G	A/A	C8	37,835,753	A/A	G/G	C9	38,155,044	G/G	T/T
C1	4,106,230	C/C	T/T	C2	4,110,442	G/G	A/A	C3	1,476,808	C/C	G/G	C3	36,798,091	G/A	A/A	C4	34,597,125	T/T	G/G	C5	9,632,050	A/A	G/G	C5	45,801,869	C/C	G/G	C7	1,248,287	T/T	C/C	C7	46,332,038	T/T	C/C	C8							

Appendix II Cont.

Table S2: *Cont.* : Differences in Allelic variants at various chromosomes positions in parent lines; the cultivated rapid cycling DHSL150 and C07060.

Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060												
C1	5,740,050	A/A	T/T	C2	4,377,234	C/C	T/T	C3	1,927,838	A/A	C/C	C3	38,989,345	A/A	C/C	C4	35,226,183	T/T	C/C	C5	9,938,185	C/C	T/T	C5	46,261,629	G/G	A/A	C7	3,542,479	A/A	G/G	C7	46,999,703	A/A	T/T	C8	38,429,505	G/G	A/A	C9	38,745,768	G/G	A/A
C1	5,754,332	T/T	G/G	C2	4,377,237	A/A	G/G	C3	1,927,853	A/A	G/G	C3	38,989,356	A/A	C/C	C4	35,227,972	C/C	T/T	C5	9,956,272	A/A	C/C	C5	46,261,650	A/A	G/G	C7	3,701,357	G/G	A/A	C7	47,003,790	G/G	A/A	C8	38,435,779	G/G	C/C	C9	38,751,225	A/A	G/G
C1	5,754,464	T/T	G/G	C2	4,568,928	G/G	A/A	C3	1,966,967	T/T	G/G	C3	38,989,376	G/G	T/T	C4	35,248,251	A/A	G/G	C5	9,956,895	A/A	C/C	C5	46,261,713	C/C	T/T	C7	3,960,363	T/T	C/C	C7	47,090,645	G/G	A/A	C8	38,436,022	C/C	T/T	C9	38,751,700	G/G	A/A
C1	5,754,508	G/G	T/T	C2	4,644,991	C/C	T/T	C3	1,980,831	G/G	T/T	C3	38,992,716	C/C	A/A	C4	35,248,277	A/A	G/G	C5	10,031,634	A/A	T/T	C5	46,325,625	C/C	A/A	C7	4,099,411	T/T	C/C	C7	47,121,669	T/T	A/A	C8	38,462,473	G/G	T/T	C9	38,751,717	C/C	T/T
C1	5,754,518	C/C	T/T	C2	5,174,132	A/A	T/T	C3	2,117,450	T/T	C/C	C3	39,037,597	T/T	C/C	C4	35,261,902	T/T	C/C	C5	10,056,072	C/C	A/A	C5	46,354,554	T/T	C/C	C7	4,531,455	T/T	C/C	C7	47,144,864	G/G	C/C	C8	38,462,512	G/G	C/C	C9	38,930,086	A/A	T/T
C1	5,792,876	A/A	G/G	C2	5,174,150	A/A	C/C	C3	2,167,225	C/C	T/T	C3	42,277,079	A/A	G/G	C4	35,285,549	A/A	T/T	C5	10,061,383	T/T	C/C	C5	46,354,564	C/C	T/T	C7	5,478,807	A/A	T/T	C7	47,147,114	G/G	A/A	C8	38,507,458	G/G	A/A	C9	38,932,054	A/A	G/G
C1	5,792,880	A/A	G/G	C2	5,230,505	G/G	A/A	C3	2,167,228	C/C	T/T	C3	42,882,678	T/T	G/G	C4	35,285,606	T/T	G/G	C5	10,314,582	G/G	T/T	C5	46,476,747	A/A	G/G	C7	5,478,819	C/C	T/T	C7	47,188,614	C/C	T/T	C8	38,507,536	T/T	C/C	C9	38,937,651	T/T	C/C
C1	5,794,717	A/A	T/T	C2	5,313,920	T/T	C/C	C3	2,213,323	G/G	A/A	C3	42,882,684	G/G	A/A	C4	35,292,615	T/T	C/C	C5	10,314,632	A/A	G/G	C5	46,482,114	A/A	G/G	C7	5,484,892	T/T	C/C	C7	47,188,626	A/A	G/G	C8	38,510,322	T/T	C/C	C9	39,171,970	T/T	C/C
C1	5,796,941	C/C	A/A	C2	5,314,220	T/T	C/C	C3	2,257,575	C/C	C/C	C3	43,621,334	T/T	C/C	C4	35,438,704	A/A	T/T	C5	10,315,863	T/T	C/C	C5	46,566,330	G/G	C/C	C7	5,484,901	T/T	A/A	C7	47,206,061	C/C	T/T	C8	38,522,923	A/A	G/G	C9	39,282,595	C/C	G/G
C1	5,797,457	G/G	C/C	C2	5,314,277	G/G	C/C	C3	2,272,459	A/A	T/T	C3	43,621,359	A/A	C/C	C4	35,503,195	G/G	C/C	C5	10,380,351	C/C	A/A	C5	46,566,612	C/C	T/T	C7	5,597,181	G/G	A/A	C7	47,253,788	T/T	C/C	C8	38,522,956	A/A	C/C	C9	39,325,484	C/C	G/G
C1	5,804,293	G/G	A/A	C2	5,348,053	C/C	T/T	C3	2,277,536	T/T	G/G	C3	43,625,558	C/C	T/T	C4	35,576,349	C/C	T/T	C5	10,444,571	T/T	A/A	C5	46,653,448	T/T	C/C	C7	5,607,286	C/C	A/A	C7	47,253,851	A/A	T/T	C8	38,523,038	G/G	A/A	C9	39,325,541	C/C	T/T
C1	5,804,306	A/A	T/T	C2	5,376,189	G/G	A/A	C3	2,277,603	G/G	A/A	C3	46,936,922	T/T	A/A	C4	35,577,587	C/C	T/T	C5	10,458,603	T/T	C/A	C5	46,654,351	G/G	T/T	C7	5,610,095	G/G	A/A	C7	47,253,878	C/C	T/T	C8	38,523,050	T/T	A/A	C9	39,325,701	C/C	G/G
C1	5,810,960	G/G	A/A	C2	5,387,068	G/G	A/A	C3	2,342,229	G/G	C/C	C3	49,923,819	T/T	C/C	C4	35,612,026	C/C	G/G	C5	10,489,039	G/G	A/A	C5	46,654,354	G/G	A/A	C7	6,394,953	G/G	A/A	C7	47,253,884	T/T	C/C	C8	38,540,608	T/T	G/G	C9	39,325,776	T/T	G/G
C1	5,811,060	G/G	C/C	C2	5,387,150	C/C	T/T	C3	2,447,850	T/T	C/C	C3	50,245,639	T/T	C/C	C4	35,612,071	A/A	G/G	C5	10,599,105	A/A	C/A	C5	46,654,387	T/T	C/C	C7	6,414,574	C/C	A/A	C7	47,288,286	G/G	C/C	C8	38,543,748	G/G	A/A	C9	39,327,652	C/C	T/T
C1	5,811,494	T/T	C/C	C2	5,447,353	T/T	C/C	C3	2,453,614	T/T	C/C	C3	50,430,761	G/G	C/C	C4	35,754,048	A/A	C/C	C5	10,706,910	G/G	C/C	C6	410,179	A/A	G/G	C7	6,431,306	T/T	C/C	C7	47,288,307	A/A	T/A	C8	38,554,687	A/A	T/T	C9	39,327,866	T/T	C/C
C1	5,811,533	C/C	T/T	C2	5,452,334	T/T	C/C	C3	2,508,636	C/C	C/C	C3	50,431,242	C/C	T/C	C4	35,754,061	A/A	G/G	C5	10,729,373	T/T	C/C	C6	412,675	A/A	G/G	C7	6,553,803	A/A	C/C	C7	47,288,427	C/C	T/A	C8	38,554,755	A/A	T/T	C9	39,327,910	A/A	T/T
C1	5,811,555	T/T	C/C	C2	5,609,425	A/A	C/C	C3	2,508,714	T/T	A/A	C3	50,813,499	A/A	T/T	C4	36,087,457	C/C	G/G	C5	10,830,338	A/A	T/T	C6	421,475	A/A	G/G	C7	6,553,816	A/A	T/T	C7	47,425,896	T/T	A/A	C8	38,560,196	G/G	A/A	C9	39,374,324	G/G	C/C
C1	5,835,848	T/T	C/C	C2	5,609,923	A/A	C/C	C3	2,535,040	G/G	A/A	C3	50,876,231	G/G	A/A	C4	36,156,570	A/A	T/T	C5	10,884,533	A/A	T/T	C6	558,722	T/T	A/A	C7	6,576,205	T/T	C/C	C7	47,626,895	C/C	T/T	C8	38,574,895	C/C	T/T	C9	39,374,361	T/T	G/G
C1	5,835,920	T/T	C/C	C2	5,610,026	G/G	T/T	C3	2,584,834	A/A	G/G	C3	50,876,263	T/T	C/C	C4	36,157,185	C/C	T/T	C5	10,884,645	C/C	T/T	C6	571,676	A/A	T/T	C7	6,576,231	C/C	T/T	C7	47,630,049	A/A	G/G	C8	38,574,985	G/G	A/A	C9	39,481,410	A/A	T/T
C1	5,899,973	C/C	T/T	C2	5,890,303	C/C	T/T	C3	2,619,544	G/G	A/A	C3	50,965,626	C/C	G/G	C4	36,157,293	T/T	A/A	C5	10,890,797	A/A	C/C	C6	672,723	T/T	C/C	C7	6,624,920	C/C	A/A	C7	47,630,058	G/G	A/A	C8	38,574,988	G/G	C/C	C9	39,494,038	A/A	G/G
C1	5,900,010	T/T	C/C	C2	5,890,337	T/T	C/C	C3	2,619,629	C/C	C/C	C3	50,965,639	G/G	A/A	C4	36,157,353	T/T	A/A	C5	10,891,500	G/G	A/A	C6	742,767	A/A	C/C	C7	6,654,266	G/G	C/C	C7	47,630,128	T/T	C/C	C8	38,635,194	C/C	G/G	C9	39,494,065	T/T	C/C
C1	5,905,754	C/C	T/T	C2	5,890,433	T/T	A/A	C3	2,749,293	C/C	T/T	C3	50,996,673	C/C	T/T	C4	36,157,805	C/A	A/A	C5	10,910,398	A/A	G/G	C6	807,989	G/G	C/C	C7	7,581,806	G/G	C/C	C7	47,630,130	C/C	T/T	C8	38,635,222	A/A	G/G	C9	39,495,100	C/C	A/A
C1	5,905,769	T/T	C/C	C2	5,905,823	T/T	A/A	C3	2,864,291	G/A	A/A	C3	51,013,364	C/C	T/T	C4	36,305,501	T/T	G/G	C5	10,910,433	T/T	G/G	C6	1,007,621	T/T	C/C	C7	7,581,812	A/A	T/T	C7	47,630,850	C/C	T/T	C8	38,635,246	A/A	G/G	C9	39,684,159	A/A	G/A
C1	5,905,901	G/G	T/T	C2	5,928,126	G/G	C/C	C3	2,866,116	T/T	A/A	C3	51,032,082	C/C	A/A	C4	36,305,504	C/C	T/T	C5	10,917,620	C/C	G/G	C6	1,007,652	A/A	C/C	C7	7,640,969	C/C	G/G	C7	47,630,895	T/T	C/C	C8	38,643,155	C/C	G/G	C9	40,225,597	A/A	G/G
C1	5,905,907	A/A	G/G	C2	5,928,133	T/T	C/C	C3	2,901,748	T/T	G/G	C3	51,099,017	T/T	G/G	C4	36,443,242	G/G	A/A	C5	10,947,608	T/T	A/A	C6	1,084,362	G/G	T/T	C7	7,752,147	A/A	G/G	C7	47,695,542	A/A	T/T	C8	38,730,772	A/A	G/G	C9	40,335,912	T/T	G/G
C1	5,909,114	T/T	C/C	C2	5,966,526	A/A	T/T	C3	2,901,762	A/A	G/G	C3	51,199,212	T/T	C/C	C4	36,443,243	T/T	C/C	C5	11,070,077	C/C	A/A	C6	1,203,501	T/T	A/A	C7	7,752,294	T/T	C/C	C7	47,695,651	T/T	C/C	C8	38,750,168	T/T	G/G	C9	40,393,636	A/A	T/T
C1	5,976,141	A/A	G/G	C2	6,110,964	A/A	G/G	C3	2,901,791	T/T	C/C	C3	51,199,229	G/G	A/A	C4	36,443,326	G/G	T/T	C5	11,070,087	G/A	C/C	C6	1,203,501	T/T	A/A	C7	7,752,384	T/T	A/A	C7	48,061,752	G/G	A/A	C8	38,750,204	C/C	T/T	C9	40,393,653	G/G	A/A
C1	5,976,159	G/G	A/A	C2	6,182,377	C/C	T/T	C3	2,945,991	G/G	C/C	C3	51,199,261	C/C	A/A	C4	36,523,586	A/A	G/G	C5	11,070,126	C/C	A/A	C6	1,203,507	C/C	C7	7,786,733	A/A	G/G	C7	48,073,603	G/G	A/A	C8	38,750,248	A/A	G/G	C9	40,393,726	G/G	T/T	
C1	5,976,201	C/C	T/T	C2	6,182,405	G/G	T/T	C3	2,968,249	G/G	T/T	C3	51,317,413	A/A	G/G	C4	36,547,603	T/T	G/G	C5	11,085,101	A/A	T/T	C6	1,203,598	T/T	G/G	C7	7,943,597	A/A	G/G	C7	48,084,305	G/G	A/A	C8	38,756,019	G/G	A/A	C9	40,438,020	T/T	C/C
C1	5,977,839	A/A	C/C	C2	6,182,431	G/G	A/A	C3	2,971,950	G/G	A/A	C3	51,317,419	T/T	A/A	C4	36,663,828	C/C	A/A	C5	11,114,829	A/A	C/C	C6	1,294,919	T/T	A/A	C7	8,000,453	A/A	T/T	C7	48,087,864	A/A	G/G	C8	38,758,146	A/A	T/T	C9	40,652,049	A/A	G/G
C1	5,977,853	T/T	G/G	C2	6,307,157	C/C	G/G	C3	3,036,810	G/G	T/T	C3	51,333,371	C/C	A/A	C4	36,663,836	T/T	C/C	C5	11,114,872	A/A	G/G	C6	1,296,351	A/A	C/C	C7	8,084,879	G/G	A/A	C7	48,087,886	G/G	A/A	C8	38,758,161	C/C	G/G	C9	40,871,822	C/C	A/A
C1	6,019,364	C/C	T/T	C2	6,307,175	G/G	A/A	C3	3,050,304	G/G	T/T	C3	51,333,388	T/T	C/C	C4	36,665,740	T/T	C/C	C5	11,119,557	C/C	G/G	C6	1,296,352	T/T	G/G	C7	8,084,898	A/A	T/T	C7	48,089,611	C/C	G/G	C8	38,838,174	A/A	G/G	C9	40,871,872	A/A	C/C
C1	6,045,864	A/A	T/T	C2	6,645,829	G/G	A/A	C3	3,050,345	G/G	A/A	C3	51,496,349	A/A	G/G	C4	36,665,929	T/T	G/G	C5	11,398,794	T/T	C/C	C6	1,296,950	A/A	G/G	C7	8,104,530	A/A	G/G	C7	48,089,617	G/G	T/T	C8	38,838,261	G/G	C/C	C9	41,045,970	A	

Appendix II *Cont.***Table S2: *Cont.* : Differences in Allelic variants at various chromosomes positions in parent lines; the cultivated rapid cycling DHSL150 and C07060.**

Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060													
C1	7,235,221	A/A	G/G	C2	8,341,785	C/C	T/T	C3	3,469,591	A/A	C/C	C3	52,967,198	A/A	G/G	C4	38,112,561	T/T	C/C	C5	13,308,754	T/T	C/C	C6	2,396,335	T/T	A/A	C7	9,984,462	A/A	G/G	C7	48,353,398	A/A	G/G	C8	39,716,176	G/G	A/A	C9	41,826,473	C/C	T/T	
C1	7,347,598	C/C	T/T	C2	8,348,852	C/C	T/T	C3	3,485,461	T/T	C/C	C3	53,004,455	A/A	A/A	T/T	C4	38,139,174	G/G	A/A	C5	13,349,061	C/C	T/T	C6	2,402,667	T/T	C/C	C7	9,985,017	C/C	G/G	C8	199,847	T/T	A/A	C8	39,716,207	A/A	T/T	C9	41,827,311	T/T	A/A
C1	7,590,658	C/C	T/T	C2	8,350,281	T/T	A/A	C3	3,489,312	T/T	C/C	C3	53,155,932	A/A	G/G	C4	38,150,634	T/T	G/G	C5	13,358,874	C/C	T/T	C6	2,402,775	A/A	T/T	C7	9,985,034	T/T	C/C	C8	284,653	C/C	T/T	C8	39,735,698	G/G	A/A	C9	41,827,365	C/C	A/A	
C1	7,638,405	T/T	A/A	C2	8,350,331	A/A	T/T	C3	3,504,765	G/G	A/A	C3	53,168,027	T/T	G/G	C4	38,178,375	G/G	A/A	C5	13,358,985	G/G	A/A	C6	2,481,011	T/T	C/C	C7	10,013,328	C/C	T/T	C8	403,383	A/A	G/G	C8	39,767,608	G/G	C/C	C9	41,940,741	C/C	T/T	
C1	7,728,530	T/T	A/A	C2	8,366,621	A/A	T/T	C3	3,546,923	A/A	C/C	C3	53,248,252	A/A	G/G	C4	38,178,387	T/T	C/C	C5	13,366,035	C/C	T/T	C6	2,616,003	C/C	A/A	C7	10,013,329	C/C	A/A	C8	403,409	A/A	G/G	C8	39,872,494	A/A	G/G	C9	41,940,816	C/C	A/A	
C1	7,746,104	A/A	G/G	C2	8,366,637	C/C	T/T	C3	3,547,025	T/T	C/C	C3	53,428,095	T/T	G/G	C4	38,196,664	C/C	G/G	C5	13,428,004	C/C	T/T	C6	2,672,090	C/C	T/T	C7	10,385,118	C/C	T/T	C8	403,410	A/A	T/T	C8	39,872,503	G/G	A/A	C9	42,061,557	C/C	A/A	
C1	7,746,117	A/A	C/C	C2	8,476,668	T/T	A/A	C3	3,575,147	T/T	C/C	C3	53,544,713	G/G	A/A	C4	38,214,765	G/G	A/A	C5	13,521,800	C/C	T/T	C6	2,747,981	A/A	G/G	C7	10,385,138	A/A	G/G	C8	416,653	C/C	T/T	C8	39,872,616	C/C	T/T	C9	42,061,593	G/G	A/A	
C1	7,746,149	A/A	C/C	C2	8,476,812	T/T	A/A	C3	3,585,288	T/T	G/G	C3	54,042,248	A/A	T/T	C4	38,214,775	C/C	A/A	C5	13,521,879	A/A	G/G	C6	2,844,806	C/C	T/T	C7	10,434,210	C/C	T/T	C8	416,656	A/A	G/G	C8	39,906,950	C/C	T/T	C9	42,081,483	A/A	G/G	
C1	8,154,945	T/T	A/A	C2	8,510,105	A/A	G/G	C3	3,585,758	A/A	G/G	C3	54,042,293	T/T	C/C	C4	38,257,559	T/T	C/C	C5	13,522,306	A/A	G/G	C6	2,844,815	G/G	A/A	C7	10,434,218	G/G	A/A	C8	416,737	G/G	A/A	C8	39,906,966	C/C	T/T	C9	42,081,594	T/T	A/A	
C1	8,155,032	A/A	G/G	C2	8,520,975	G/G	T/T	C3	3,585,787	T/T	A/A	C3	54,295,387	C/C	T/T	C4	38,270,051	C/C	T/T	C5	13,646,750	A/A	C/C	C6	2,917,386	C/C	T/T	C7	10,434,271	T/T	G/G	C8	458,519	C/C	A/A	C8	39,964,758	G/G	C/C	C9	42,081,600	T/T	G/G	
C1	8,155,987	A/A	G/G	C2	8,521,071	T/T	C/C	C3	3,718,265	T/T	C/C	C3	54,325,517	C/C	T/T	C4	38,474,756	C/C	T/T	C5	13,822,410	A/A	G/G	C6	3,067,641	A/A	G/G	C7	10,601,447	A/A	C/A	C8	458,743	C/C	T/T	C8	40,004,725	T/T	C/C	C9	42,081,633	T/T	C/C	
C1	8,186,080	C/C	G/G	C2	8,540,048	T/T	C/C	C3	3,722,478	T/T	A/A	C3	54,403,845	T/T	C/C	C4	38,482,963	A/A	C/C	C5	13,831,598	A/A	G/G	C6	3,072,991	T/T	A/A	C7	10,612,303	G/G	A/A	C8	458,766	G/G	A/A	C8	40,135,075	G/G	A/A	C9	42,085,548	C/C	T/T	
C1	8,276,217	C/C	T/T	C2	8,581,072	G/G	A/A	C3	3,963,328	T/T	A/A	C3	54,403,990	T/T	C/C	C4	38,511,586	C/C	G/G	C5	13,831,608	A/A	C/C	C6	3,215,453	T/T	A/A	C7	10,612,369	A/A	G/G	C8	461,449	G/G	C/C	C8	40,135,128	T/T	C/C	C9	42,131,020	A/A	C/C	
C1	8,276,291	T/T	A/A	C2	8,581,088	T/T	G/G	C3	3,973,894	T/T	C/C	C3	54,404,064	G/G	C/C	C4	38,571,450	C/C	T/T	C5	13,831,654	T/T	C/C	C6	3,215,611	T/T	C/C	C7	10,612,409	G/G	A/A	C8	461,944	C/C	A/A	C8	40,135,146	A/A	C/C	C9	42,420,885	G/G	A/A	
C1	8,325,018	C/C	T/T	C2	8,612,789	C/C	A/A	C3	4,011,152	C/C	T/T	C3	54,619,326	T/T	C/C	C4	38,692,508	G/G	A/A	C5	13,831,694	T/T	C/C	C6	3,299,494	G/G	A/A	C7	10,953,400	A/A	C/A	C8	468,240	A/A	T/T	C8	40,142,147	A/A	G/G	C9	42,421,432	A/A	G/G	
C1	8,325,899	T/T	G/G	C2	8,612,830	A/A	C/C	C3	4,078,381	G/G	A/A	C3	55,027,956	C/C	T/T	C4	39,025,815	G/G	C/C	C5	13,831,731	A/A	G/G	C6	3,553,036	T/T	C/C	C7	10,958,100	C/C	G/G	C8	473,902	G/G	T/T	C8	40,142,539	G/G	C/C	C9	42,421,485	A/A	G/G	
C1	8,354,702	G/G	A/A	C2	8,620,014	A/A	T/T	C3	4,078,412	G/G	A/A	C3	55,027,996	G/G	A/A	C4	39,030,920	A/A	G/G	C5	13,992,354	G/G	A/A	C6	3,596,863	C/C	T/T	C7	11,508,072	G/G	A/A	C8	496,081	G/G	A/A	C8	40,236,102	T/T	C/C	C9	42,550,068	G/G	A/A	
C1	8,357,698	G/G	C/C	C2	8,620,039	A/A	T/T	C3	4,103,549	G/G	A/A	C3	55,028,011	A/A	G/G	C4	39,057,539	G/G	A/A	C5	14,000,504	A/A	G/G	C6	3,653,701	T/T	C/C	C7	11,509,127	T/T	A/A	C8	496,129	G/G	A/A	C8	40,236,116	C/C	C/C	C9	42,587,589	A/A	T/T	
C1	8,357,757	T/T	C/C	C2	8,620,054	A/A	G/G	C3	4,112,214	C/C	G/G	C3	55,088,046	G/A	G/G	C4	39,085,888	A/A	G/G	C5	14,058,294	C/C	T/T	C6	3,728,888	A/A	G/G	C7	11,513,293	G/G	T/A	C8	524,775	T/T	C/C	C8	40,236,117	T/T	C/C	C9	42,636,417	A/A	T/T	
C1	8,357,784	G/G	A/A	C2	8,620,092	A/A	G/G	C3	4,511,705	T/T	G/G	C3	55,089,392	C/C	T/T	C4	39,126,526	C/C	T/T	C5	14,237,089	C/C	A/A	C6	3,734,096	A/A	G/G	C7	11,513,961	C/C	A/A	C8	524,788	G/G	A/A	C8	40,302,362	A/A	G/G	C9	42,637,020	T/T	G/G	
C1	8,357,849	C/C	A/A	C2	8,620,102	A/A	G/G	C3	4,511,706	A/A	G/G	C3	55,270,126	A/A	G/G	C4	39,126,536	T/T	C/C	C5	14,237,691	T/T	C/C	C6	3,829,212	C/C	T/T	C7	11,514,597	C/C	T/A	C8	590,138	C/C	T/T	C8	40,304,550	G/G	A/A	C9	42,648,594	A/A	G/G	
C1	8,357,863	A/A	C/C	C2	8,627,938	C/C	T/T	C3	4,515,732	A/A	C/C	C3	55,789,071	C/C	T/T	C4	39,126,538	G/G	C/C	C5	14,325,237	T/T	C/C	C6	3,829,294	G/G	C/C	C7	11,514,660	T/T	A/A	C8	663,150	T/T	C/C	C8	40,324,333	G/G	A/A	C9	42,828,194	A/A	C/C	
C1	8,360,755	C/C	G/G	C2	8,655,159	G/G	T/T	C3	4,516,851	A/A	G/G	C3	55,927,487	A/A	G/G	C4	39,733,707	T/T	C/C	C5	14,381,619	G/G	C/C	C6	3,969,299	A/A	G/G	C7	11,534,944	C/C	T/T	C8	663,163	C/C	A/A	C8	40,326,135	T/T	A/A	C9	42,828,236	A/A	C/C	
C1	8,361,568	T/T	G/G	C2	8,655,204	T/T	G/G	C3	4,516,871	G/G	C/C	C3	56,616,124	C/C	A/A	C4	39,737,798	G/G	C/C	C5	14,381,634	T/T	C/C	C6	4,096,072	A/A	G/G	C7	11,536,176	C/C	G/G	C8	663,326	G/G	A/A	C8	40,326,187	T/T	C/C	C9	42,866,792	G/G	C/C	
C1	8,361,595	A/A	G/G	C2	8,658,346	T/T	A/A	C3	4,516,959	G/G	A/A	C3	57,137,182	G/G	A/A	C4	39,827,816	T/T	C/C	C5	14,505,824	C/C	C/C	C6	4,096,094	G/G	A/A	C7	12,233,354	A/A	T/T	C8	671,730	C/C	T/T	C8	40,326,187	G/G	T/T	C9	42,866,897	G/G	A/A	
C1	8,361,640	C/C	T/T	C2	8,658,408	A/A	T/T	C3	4,528,250	G/G	A/A	C3	57,161,906	C/C	T/T	C4	39,836,018	A/A	C/C	C5	14,508,678	T/T	T/T	C6	4,096,178	C/C	A/A	C7	12,239,433	T/T	G/G	C8	1,235,590	G/G	A/A	C8	40,328,271	G/G	A/A	C9	42,869,545	C/C	T/T	
C1	8,361,703	C/C	T/T	C2	8,658,446	A/A	G/G	C3	4,552,099	G/G	A/A	C3	57,241,520	C/C	T/T	C4	39,836,036	G/G	A/A	C5	14,644,755	G/G	C/C	C6	4,123,436	T/T	G/G	C7	12,239,944	G/G	T/T	C8	1,246,113	A/A	C/C	C8	40,335,558	C/C	T/T	C9	42,870,122	G/G	A/A	
C1	8,361,729	G/G	A/A	C2	8,726,562	G/G	A/A	C3	4,596,108	A/A	G/G	C3	57,266,646	A/A	C/C	C4	39,854,739	T/T	G/G	C5	14,842,340	G/G	A/A	C6	4,256,436	T/T	G/G	C7	12,240,955	C/C	T/T	C8	1,265,426	C/C	A/A	C8	40,335,650	G/G	A/A	C9	42,933,619	G/G	A/A	
C1	8,402,822	A/A	G/G	C2	8,739,255	C/C	A/A	C3	4,633,791	T/T	A/A	C3	57,392,433	C/C	T/T	C4	39,854,740	T/T	A/A	C5	14,842,361	A/A	G/G	C6	4,315,718	A/A	C/C	C7	12,257,822	T/T	G/G	C8	1,279,468	C/C	A/A	C8	40,336,377	T/T	C/C	C9	42,955,146	C/C	G/G	
C1	8,568,254	T/T	C/C	C2	8,805,114	T/T	C/C	C3	4,636,473	A/A	G/G	C3	57,630,333	C/C	T/T	C4	39,885,999	T/T	G/G	C5	15,209,252	C/A	A/A	C6	4,315,792	A/A	G/G	C7	12,257,832	G/G	C/C	C8	1,279,477	T/T	C/C	C8	40,336,531	C/C	T/T	C9	42,955,175	G/G	A/A	
C1	8,658,416	C/C	T/T	C2	9,214,784	G/G	C/C	C3	4,708,392	T/T	G/G	C3	57,630,392	T/T	C/C	C4	39,886,084	T/T	A/A	C5	15,209,775	A/A	T/T	C6	4,739,896	A/A	T/T	C7	12,257,835	C/C	A/A	C8	1,279,504	G/G	A/A	C8	40,390,509	T/T	G/G	C9	42,955,240	T/T	C/C	
C1	8,658,434	C/C	T/T	C2	9,246,694	A/A	G/G	C3	4,708,427	A/A	G/G	C3	57,926,687	A/A	G/G	C4	39,920,355	G/G	C/C	C5	15,209,785	A/A	T/T	C6	4,749,751	T/T	A/A	C7	12,258,392	T/T	C/C	C8	1,279,573	A/A	G/G	C8	40,452,074	T/T	G/G	C9	42,955,257	T/T	C/C	
C1	8,742,500	C/C	T/T	C2	9,246,780	A/A	G/G	C3	4,714,786	A/A	G/G	C3	58,193,621	G/G	A/A	C4	39,920,430	G/G	A/A	C5	15,209,874	A/A	G/G	C6	4,749,776	C/C	T/T	C7	12,283,411	A/A	G/G	C8	1,279,641	G/G	A/A	C8	40,452,090	C/C	T/T	C9	42,966,773	A/A	C/C	
C1	8																																											

Appendix II Cont.

Table S2: Cont. : Differences in Allelic variants at various chromosomes positions in parent lines; the cultivated rapid cycling DHSL150 and C07060.

Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060												
C1	9,489,962	C/C	T/T	C2	9,364,051	C/C	T/T	C3	4,820,860	T/T	C/C	C3	59,723,653	C/C	T/T	C4	40,379,101	C/C	T/T	C5	15,788,266	C/C	A/A	C6	5,634,507	A/A	G/G	C7	13,540,266	T/T	G/G	C8	1,960,191	C/C	T/T	C8	40,675,322	A/A	G/G	C9	43,310,437	A/A	C/C
C1	9,490,001	A/A	T/T	C2	9,371,718	C/C	T/T	C3	4,820,872	T/T	A/A	C3	59,748,791	T/T	C/C	C4	40,413,160	T/T	G/G	C5	15,806,738	G/G	A/A	C6	5,730,719	A/A	G/G	C7	13,651,810	A/A	T/T	C8	1,994,897	A/A	G/G	C8	40,677,578	T/T	G/G	C9	43,310,532	T/T	C/C
C1	9,511,838	A/A	T/T	C2	9,391,977	T/T	A/A	C3	4,878,143	G/G	A/A	C3	60,079,122	A/A	G/G	C4	40,416,513	C/C	A/A	C5	15,806,780	A/A	T/T	C6	6,138,049	G/G	A/A	C7	13,862,645	A/A	G/G	C8	1,996,866	T/T	G/G	C8	40,733,163	G/G	T/T	C9	43,335,901	A/A	G/G
C1	9,511,852	C/C	T/T	C2	9,391,994	A/A	G/G	C3	4,965,040	C/C	T/T	C3	60,079,187	C/C	T/T	C4	40,476,309	A/A	G/G	C5	15,808,505	T/T	C/C	C6	6,288,174	G/G	C/C	C7	13,870,555	T/T	G/G	C8	2,066,862	A/A	T/T	C8	40,733,214	C/C	T/T	C9	43,858,243	T/T	A/A
C1	9,522,073	G/G	A/G	C2	9,846,293	T/T	C/C	C3	5,066,086	C/C	T/T	C3	60,182,206	T/T	C/C	C4	40,476,333	G/G	A/A	C5	15,904,375	T/T	G/G	C6	6,330,290	A/A	T/T	C7	13,870,606	T/T	C/C	C8	2,085,890	C/A	A/A	C8	40,780,307	G/G	A/A	C9	44,254,819	T/T	C/C
C1	9,524,397	A/A	G/G	C2	9,933,345	T/T	C/C	C3	5,066,088	A/A	G/G	C3	60,189,670	T/T	C/C	C4	40,705,740	A/A	G/G	C5	16,040,858	T/T	A/A	C6	6,360,904	G/G	T/T	C7	13,912,769	G/G	A/A	C8	2,085,909	T/T	G/G	C8	40,780,310	T/T	A/A	C9	44,359,776	G/G	T/T
C1	9,524,412	A/A	T/T	C2	10,079,709	T/T	C/C	C3	5,066,107	T/T	C/C	C3	60,227,681	C/T	A/A	C4	40,705,782	T/T	C/C	C5	16,078,191	T/T	C/C	C6	6,435,269	G/G	A/A	C7	13,914,678	T/T	A/A	C8	2,100,087	A/A	T/T	C8	40,782,341	G/G	C/C	C9	44,400,567	T/T	C/C
C1	9,524,462	A/A	C/C	C2	10,245,718	G/G	A/A	C3	5,067,999	A/A	G/G	C3	60,232,906	A/G	G/G	C4	40,871,691	A/A	G/G	C5	16,105,469	C/C	T/T	C6	6,444,888	G/G	T/T	C7	13,926,285	A/A	G/G	C8	2,100,109	C/T	T/T	C8	40,875,431	T/T	A/A	C9	44,408,678	A/A	G/G
C1	9,638,680	G/G	T/T	C2	10,277,286	A/A	C/C	C3	5,070,201	T/T	C/C	C3	60,474,786	G/G	C/C	C4	40,874,070	A/A	G/G	C5	16,105,483	G/G	T/T	C6	6,445,442	G/G	T/T	C7	14,075,963	T/T	C/C	C8	2,113,590	T/T	G/G	C8	40,914,306	G/G	A/A	C9	44,408,887	G/G	A/A
C1	9,801,794	T/T	G/G	C2	10,277,291	C/C	T/T	C3	5,073,749	A/A	C/C	C3	60,518,901	A/A	C/C	C4	40,877,853	C/C	T/T	C5	16,247,592	T/T	G/G	C6	6,445,448	A/A	C/C	C7	14,278,053	G/G	T/T	C8	2,113,614	A/A	T/T	C8	40,939,928	T/T	C/C	C9	44,546,183	T/T	C/C
C1	10,181,302	T/T	G/G	C2	10,277,305	C/C	A/A	C3	5,073,751	G/G	A/A	C3	60,557,115	T/T	C/C	C4	41,082,277	A/A	G/G	C5	16,247,603	T/T	C/C	C6	6,445,488	G/G	A/A	C7	14,278,064	G/G	A/A	C8	2,729,246	C/T	T/T	C8	40,944,425	G/G	A/A	C9	44,546,208	A/A	G/G
C1	10,294,107	T/T	A/A	C2	10,360,938	G/G	C/C	C3	5,098,751	G/G	A/A	C3	60,557,177	C/T	T/T	C4	41,082,304	T/T	G/G	C5	16,248,743	T/T	C/C	C6	6,492,543	C/C	T/T	C7	14,637,668	G/G	A/A	C8	3,021,900	C/C	T/T	C8	40,944,432	T/T	C/C	C9	44,579,468	C/C	T/T
C1	10,512,076	C/C	T/T	C2	10,361,037	G/G	G/G	C3	5,098,830	G/G	T/T	C3	60,619,557	A/G	G/G	C4	41,573,232	T/T	C/C	C5	16,292,336	T/T	A/A	C6	6,566,228	T/T	A/A	C7	16,947,925	G/G	C/C	C8	3,338,306	G/G	A/A	C8	40,944,512	A/A	T/T	C9	44,850,724	T/T	C/C
C1	10,512,135	T/T	G/G	C2	10,392,737	T/T	A/A	C3	5,099,294	C/A	A/A	C3	60,619,726	A/A	G/G	C4	41,617,944	T/T	G/G	C5	16,292,413	T/T	C/C	C6	6,658,992	G/G	A/A	C7	16,948,307	A/A	T/T	C8	3,373,760	C/A	A/A	C8	41,070,736	T/T	A/A	C9	44,890,829	T/T	C/C
C1	10,512,139	C/C	G/G	C2	10,392,762	C/T	T/T	C3	5,099,332	T/T	A/A	C3	60,619,733	T/T	C/C	C4	41,617,965	A/A	G/G	C5	16,292,441	A/A	G/G	C6	6,718,530	G/G	A/A	C7	17,115,800	C/A	T/T	C8	3,412,352	C/T	T/T	C8	41,081,447	G/G	A/A	C9	44,984,162	G/G	A/A
C1	10,543,906	G/G	A/A	C2	10,392,838	T/T	A/A	C3	5,099,356	A/A	G/G	C3	60,640,707	G/G	A/A	C4	41,618,031	C/C	T/T	C5	16,321,442	C/C	T/T	C6	6,720,103	T/T	A/A	C7	17,134,765	A/A	C/C	C8	3,442,987	A/A	C/C	C8	41,089,700	C/C	T/T	C9	44,984,391	A/A	G/G
C1	10,584,662	T/T	C/C	C2	10,392,862	G/G	A/A	C3	5,099,369	G/G	C/C	C3	60,642,316	A/A	T/T	C4	41,636,206	G/G	A/A	C5	16,321,444	C/C	A/A	C6	6,720,130	G/G	A/A	C7	17,295,485	G/G	T/T	C8	3,445,976	A/A	G/G	C8	41,089,766	G/G	A/A	C9	44,984,393	T/T	A/A
C1	10,595,145	A/A	C/C	C2	10,400,922	A/A	C/C	C3	5,206,895	G/G	A/A	C3	60,642,501	C/C	C/C	C4	41,637,553	A/A	G/G	C5	16,321,481	A/A	G/G	C6	6,726,970	A/A	T/T	C7	17,295,638	T/T	C/C	C8	3,545,001	T/T	C/C	C8	41,089,778	C/C	T/T	C9	45,143,152	T/T	G/G
C1	10,595,148	G/G	A/A	C2	10,518,010	G/G	C/C	C3	5,206,918	C/C	T/T	C3	60,999,748	C/C	T/T	C4	41,707,163	T/T	C/C	C5	16,334,063	A/A	G/G	C6	6,931,401	G/G	T/T	C7	17,339,656	G/G	T/T	C8	3,553,585	T/T	A/A	C8	41,101,119	G/G	C/C	C9	45,143,172	T/T	C/C
C1	10,595,254	T/T	A/A	C2	10,518,661	C/C	T/T	C3	5,228,470	A/A	G/G	C3	61,001,058	C/C	T/T	C4	41,816,429	T/T	C/C	C5	16,334,100	G/G	T/T	C6	7,044,936	G/G	T/T	C7	17,449,193	A/A	C/C	C8	3,555,441	T/T	A/A	C8	41,241,303	G/G	C/C	C9	45,143,199	G/G	C/C
C1	10,595,296	A/A	G/G	C2	10,518,663	A/A	C/C	C3	5,230,181	G/G	A/A	C3	61,001,074	C/C	T/T	C4	41,816,471	A/A	C/C	C5	16,364,864	A/A	C/C	C6	7,091,782	A/A	C/C	C7	17,449,247	A/A	G/G	C8	3,555,472	C/A	A/A	C8	41,241,993	C/C	T/T	C9	45,147,021	A/A	C/C
C1	10,595,494	C/C	T/T	C2	10,519,046	A/A	G/G	C3	5,230,202	G/G	A/A	C3	61,001,101	G/G	T/T	C4	41,816,477	A/A	C/C	C5	16,431,763	T/T	G/G	C6	7,204,238	A/A	C/A	C7	17,462,582	C/C	T/T	C8	3,555,516	C/C	T/T	C8	41,242,019	G/G	A/A	C9	45,222,015	C/C	G/G
C1	10,597,099	G/G	A/A	C2	10,676,299	C/C	T/T	C3	5,310,311	C/C	A/A	C3	61,071,942	T/T	C/C	C4	41,839,863	T/T	C/C	C5	16,478,896	G/G	T/T	C6	7,204,297	A/A	C/A	C7	18,323,602	G/G	A/A	C8	3,607,808	G/G	A/A	C8	41,242,081	T/T	G/G	C9	45,222,041	T/T	C/C
C1	10,597,235	C/C	A/A	C2	10,677,830	G/G	C/C	C3	5,393,187	C/C	T/T	C3	61,071,972	C/T	A/A	C4	42,290,346	G/G	A/A	C5	16,679,059	C/C	T/T	C6	7,204,435	T/T	C/C	C7	18,389,969	G/G	A/A	C8	3,648,252	G/G	A/A	C8	41,271,383	A/A	G/G	C9	45,257,629	A/A	T/T
C1	10,597,240	A/A	G/G	C2	11,092,465	A/A	T/T	C3	5,393,236	A/A	T/T	C3	61,071,973	C/T	A/A	C4	42,290,401	C/T	T/T	C5	16,724,647	G/G	A/A	C6	7,260,812	G/G	C/C	C7	18,390,000	G/G	C/C	C8	3,698,043	A/A	C/C	C8	41,277,197	A/A	G/G	C9	45,262,021	C/A	A/A
C1	10,597,252	T/T	C/C	C2	11,098,208	T/T	C/C	C3	5,525,794	A/A	C/C	C3	61,072,071	G/G	A/A	C4	42,442,052	T/T	A/A	C5	16,812,585	A/A	G/G	C6	7,515,277	G/G	G/G	C7	18,425,984	G/G	C/C	C8	3,817,300	T/T	C/C	C8	41,396,963	G/G	A/A	C9	45,304,371	T/T	G/G
C1	11,087,823	C/C	G/G	C2	11,122,009	G/G	A/A	C3	5,525,018	G/G	A/A	C3	61,104,795	G/G	A/A	C4	42,508,207	G/G	A/A	C5	16,813,987	A/A	G/G	C6	7,544,192	T/T	A/A	C7	18,524,473	T/T	G/G	C8	3,817,304	G/G	A/A	C8	41,397,044	A/A	G/G	C9	45,312,817	C/C	T/T
C1	11,101,901	G/G	A/A	C2	11,165,693	A/A	G/G	C3	5,526,056	G/G	A/A	C3	61,104,807	G/G	T/T	C4	42,508,213	C/C	T/T	C5	16,867,642	C/C	G/G	C6	7,544,266	C/C	G/G	C7	18,524,508	T/T	A/A	C8	3,828,804	C/C	T/T	C8	41,419,201	C/C	G/G	C9	45,523,800	G/G	A/A
C1	11,214,670	G/G	A/A	C2	11,294,914	C/C	T/T	C3	5,614,902	G/A	A/A	C3	61,107,111	C/C	T/T	C4	42,508,234	G/G	A/A	C5	16,867,684	C/C	G/G	C6	7,549,142	C/A	A/A	C7	18,728,818	A/A	G/A	C8	3,854,869	C/C	T/T	C8	41,419,220	C/C	G/G	C9	45,523,812	T/T	A/A
C1	11,216,197	T/T	G/G	C2	11,383,354	C/C	G/G	C3	5,658,031	G/G	T/T	C3	61,107,139	A/A	G/G	C4	42,508,327	G/G	C/C	C5	16,867,738	A/A	T/T	C6	7,549,702	C/C	T/T	C7	18,728,953	T/T	C/C	C8	4,221,603	A/A	G/G	C8	41,546,990	T/T	A/A	C9	45,533,682	A/A	G/G
C1	11,216,323	G/G	A/A	C2	11,383,423	A/A	G/G	C3	5,684,044	G/G	A/A	C4	61,107,162	G/G	A/A	C4	42,508,345	G/G	C/C	C5	16,871,400	A/A	G/G	C6	7,549,766	G/G	C/C	C7	18,816,116	A/A	C/C	C8	4,401,105	A/A	C/C	C8	41,547,016	G/G	A/A	C9	45,533,916	A/A	G/G
C1	11,238,171	T/T	C/C	C2	11,383,435	G/G	A/A	C3	5,801,734	A/A	T/T	C3	61,107,170	G/G	A/A	C4	42,525,980	C/C	A/A	C5	17,178,870	C/A	C/C	C6	7,659,273	T/T	C/C	C7	18,816,170	C/C	T/T	C8	4,401,229	A/A	G/G	C8	41,547,018	C/A	A/A	C9	45,559,406	T/T	C/C
C1	11,408,542	C/C	T/T	C2	11,387,857	C/C	A/A	C3	5,860,860	T/T	A/A	C4	61,107,178	C/C	A/A	C4	42,644,290	C/C	A/A	C5	17,290,150	A/A	G/G	C6	7,687,819	A/A	G/G	C7	19,344,240	C/A	G/G	C8	4,492,855	A/A	C								

Appendix II Cont.

Table S2: *Cont.* : Differences in Allelic variants at various chromosomes positions in parent lines; the cultivated rapid cycling DHSL150 and C07060.

Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060												
C1	12,597,941	C/C		C2	12,628,958	A/A	G/G	C3	6,369,303	G/G	T/T	C3	62,235,387	A/A	G/G	C4	44,564,134	G/G	T/T	C5	20,113,649	G/G	C/C	C6	9,146,934	A/A	G/G	C7	23,956,053	A/A	G/G	C8	5,772,045	A/A	C/C	C9	460,993	A/A	G/G	C9	46,356,624	A/A	G/G
C1	12,732,583	G/G	A/A	C2	12,628,963	A/A	G/G	C3	6,369,429	T/T	A/A	C3	62,235,409	C/C	T/T	C4	44,730,289	T/T	C/C	C5	20,127,424	T/T	G/G	C6	9,365,341	T/T	C/C	C7	23,956,146	A/A	C/C	C8	5,795,980	G/G	C/C	C9	461,011	C/C	G/G	C9	46,417,240	A/A	G/G
C1	12,734,905	G/G	A/A	C2	12,629,020	C/C	T/T	C3	6,383,243	T/T	C/C	C3	62,235,412	T/T	C/C	C4	44,730,397	C/C	T/T	C5	20,130,786	A/A	T/T	C6	9,384,960	G/G	T/T	C7	23,956,211	A/A	G/G	C8	5,913,865	A/A	T/T	C9	461,026	T/T	C/C	C9	46,417,336	A/A	C/C
C1	12,734,950	G/G	A/A	C2	12,629,050	T/T	C/C	C3	6,383,317	A/A	G/G	C3	62,235,459	G/G	A/A	C4	44,803,812	C/C	A/A	C5	20,130,835	T/T	G/G	C6	9,394,496	C/C	T/T	C7	23,956,312	A/A	G/G	C8	5,917,228	G/G	A/A	C9	461,080	T/T	C/C	C9	46,522,556	C/C	T/T
C1	12,734,956	C/C	T/T	C2	12,629,062	T/T	C/C	C3	6,383,383	C/C	T/T	C4	62,312,888	C/C	T/T	C4	44,803,850	C/C	T/T	C5	20,158,137	G/G	C/C	C6	9,394,506	C/C	T/T	C7	24,039,370	T/T	A/A	C8	5,963,178	A/A	T/T	C9	510,525	A/A	G/G	C9	46,522,559	T/T	G/G
C1	12,770,133	G/G	T/T	C2	12,665,538	T/T	C/C	C3	6,383,413	C/C	T/T	C3	62,322,200	C/C	T/T	C4	45,208,238	T/T	C/C	C5	20,158,159	A/A	C/C	C6	9,400,019	T/T	A/A	C7	24,147,978	G/G	A/A	C8	5,963,195	C/C	T/T	C9	515,690	T/T	G/G	C9	46,779,806	G/G	T/T
C1	12,770,186	G/G	T/T	C2	12,665,691	G/G	A/A	C3	6,413,427	A/A	C/C	C3	62,322,290	A/A	G/G	C4	45,319,494	T/T	C/C	C5	20,166,636	G/G	A/A	C6	9,400,026	T/T	G/G	C7	24,148,001	T/T	G/G	C8	6,156,753	T/T	A/A	C9	534,744	C/C	T/T	C9	46,841,513	C/C	T/T
C1	12,770,243	A/A	T/T	C2	12,691,455	G/G	C/C	C3	6,415,834	C/C	T/T	C3	62,434,364	G/G	A/A	C4	45,389,693	C/C	G/G	C5	20,249,695	T/T	G/G	C6	9,606,306	A/A	C/C	C7	24,405,246	G/G	A/A	C8	6,234,934	T/T	A/A	C9	534,797	A/A	G/G	C9	46,841,707	C/C	A/A
C1	12,771,390	T/T	G/G	C2	12,691,482	A/A	C/C	C3	6,447,910	A/A	C/C	C3	62,434,405	G/G	C/C	C4	45,389,714	G/G	A/A	C5	20,249,807	G/G	A/A	C6	9,632,753	A/A	C/C	C7	24,405,314	C/C	T/T	C8	6,451,981	A/A	G/G	C9	541,090	C/C	T/T	C9	46,841,741	C/A	A/A
C1	12,771,422	G/G	A/A	C2	12,691,495	G/G	T/T	C3	6,452,903	T/T	A/A	C3	62,557,276	C/C	A/A	C4	45,576,026	A/A	G/G	C5	20,474,185	T/T	G/G	C6	9,695,628	A/A	T/T	C7	24,405,354	G/G	A/A	C8	6,455,172	A/A	G/G	C9	564,636	C/C	A/A	C9	46,841,745	T/T	A/A
C1	12,771,440	A/A	T/T	C2	12,694,393	T/T	C/C	C3	6,477,762	T/T	C/C	C3	62,785,489	A/A	G/G	C4	45,578,105	T/T	G/G	C5	20,524,666	C/C	T/T	C6	9,696,173	C/C	T/T	C7	24,406,530	G/G	A/A	C8	6,509,364	G/G	A/A	C9	594,410	T/T	C/C	C9	46,846,868	C/C	T/T
C1	13,199,262	A/A	T/T	C2	12,694,402	C/C	T/T	C3	6,538,761	G/G	A/A	C3	62,787,467	T/T	C/C	C4	45,578,383	A/A	G/G	C5	20,525,246	T/T	A/A	C6	9,696,186	G/G	T/T	C7	24,406,545	G/G	C/C	C8	6,533,118	T/T	A/A	C9	594,427	G/G	A/A	C9	46,859,807	A/A	T/T
C1	13,310,991	A/A	C/C	C2	12,694,413	C/C	T/T	C3	6,689,051	C/G	G/G	C3	62,797,070	T/T	C/C	C4	45,886,336	G/G	A/A	C5	20,525,280	T/T	A/A	C6	9,696,216	C/C	T/T	C7	24,406,607	T/T	C/C	C8	6,626,835	T/T	C/C	C9	861,945	G/G	A/A	C9	46,859,820	A/A	C/C
C1	13,323,509	C/C	G/G	C2	12,720,551	T/T	T/T	C3	6,689,192	T/T	G/G	C3	62,797,099	A/A	G/G	C4	45,979,779	T/T	A/A	C5	20,525,285	T/T	C/C	C6	9,699,354	T/T	A/A	C7	24,434,576	G/G	C/C	C8	6,626,926	G/G	C/C	C9	866,043	C/C	A/A	C9	46,859,833	C/C	T/T
C1	13,323,516	T/T	A/A	C2	12,730,319	T/T	C/C	C3	6,743,587	G/G	A/A	C3	62,797,122	T/T	A/A	C4	45,979,879	C/C	A/A	C5	20,600,720	T/T	C/C	C6	9,735,853	G/G	A/A	C7	24,434,590	A/A	G/G	C8	6,669,784	T/T	C/C	C9	889,800	A/A	G/G	C9	46,964,852	C/C	T/T
C1	13,486,371	C/C	A/A	C2	12,805,459	A/A	G/G	C3	6,825,544	T/T	G/G	C3	62,798,933	A/A	G/G	C4	45,979,890	G/G	T/T	C5	20,600,760	G/G	T/T	C6	9,736,644	C/C	T/T	C7	24,434,646	G/G	A/A	C8	6,979,975	T/T	G/G	C9	920,957	C/C	A/A	C9	47,001,087	G/G	T/T
C1	13,652,615	C/C	T/T	C2	13,038,647	A/A	T/T	C3	6,834,299	T/T	C/C	C3	62,798,952	A/A	G/G	C4	45,979,932	T/T	C/C	C5	20,711,078	C/C	T/T	C6	9,737,283	C/C	G/G	C7	24,435,601	G/G	T/T	C8	6,979,992	G/G	A/A	C9	920,967	G/G	A/A	C9	47,100,165	G/G	A/A
C1	13,818,430	G/G	A/A	C2	13,038,708	T/T	G/G	C3	6,834,387	T/T	C/C	C3	62,798,995	G/G	A/A	C4	46,057,986	C/C	T/T	C5	20,711,247	G/G	A/A	C6	9,738,059	A/A	C/C	C7	24,435,626	C/C	T/T	C8	6,980,055	T/T	C/C	C9	923,398	T/T	C/C	C9	47,110,445	A/A	C/C
C1	14,074,692	C/C	G/G	C2	13,180,853	A/A	G/G	C3	6,899,953	C/C	T/T	C3	62,941,712	A/A	T/T	C4	46,097,093	C/C	T/T	C5	20,711,265	A/A	G/G	C6	9,738,177	A/A	G/G	C7	24,435,693	A/A	G/G	C8	7,089,690	T/T	C/A	C9	924,428	G/G	A/A	C9	47,228,425	T/T	C/C
C1	14,074,741	C/C	T/T	C2	13,222,399	T/T	A/A	C3	6,899,959	G/G	A/A	C3	63,025,357	T/T	G/G	C4	46,136,636	A/A	G/G	C5	20,711,308	C/C	T/T	C6	9,738,181	A/A	T/T	C7	24,489,428	A/A	G/G	C8	7,165,130	A/A	G/G	C9	924,518	A/A	G/G	C9	47,228,441	T/T	C/C
C1	14,120,307	C/C	T/T	C2	13,222,404	C/C	T/T	C3	6,903,114	A/A	G/G	C3	63,127,865	C/C	T/T	C4	46,136,643	T/T	C/C	C5	20,758,302	A/A	C/C	C6	9,738,382	A/A	C/C	C7	24,489,455	G/G	A/A	C8	7,166,209	A/A	G/G	C9	924,561	C/C	A/A	C9	47,228,507	T/T	C/C
C1	14,187,285	A/A	T/T	C2	13,222,424	A/A	G/G	C3	6,903,115	G/G	A/A	C3	63,144,980	G/G	A/A	C4	46,137,192	C/C	T/T	C5	20,852,303	C/C	T/T	C6	9,788,551	G/G	A/A	C7	24,489,538	T/T	C/C	C8	7,166,223	T/T	C/C	C9	934,063	T/T	A/A	C9	47,269,490	T/T	C/C
C1	14,187,293	C/C	T/T	C2	13,222,454	C/C	T/T	C3	6,903,135	T/T	C/C	C3	63,254,600	C/C	T/T	C4	46,137,227	T/T	C/C	C5	21,109,741	C/C	T/T	C6	9,788,778	A/A	T/T	C7	24,489,599	T/T	A/A	C8	7,167,668	C/C	T/T	C9	948,104	C/C	G/G	C9	47,269,493	C/C	G/G
C1	14,230,334	T/T	C/C	C2	13,617,193	T/T	A/A	C3	6,960,773	A/A	G/G	C3	63,279,051	C/C	T/T	C4	46,137,249	T/T	C/C	C5	21,109,758	T/T	C/C	C6	9,788,793	C/C	T/T	C7	24,489,709	G/G	T/T	C8	7,243,991	G/G	T/T	C9	948,109	A/A	G/G	C9	47,269,679	A/A	G/G
C1	14,230,337	T/T	A/A	C2	13,617,248	A/A	G/G	C3	7,043,300	A/A	G/G	C3	63,279,489	T/T	A/A	C4	46,137,270	C/C	G/G	C5	21,166,445	G/G	A/A	C6	9,819,559	A/A	C/C	C7	24,638,931	A/A	C/C	C8	7,365,223	T/T	A/A	C9	989,775	C/C	G/G	C9	47,269,703	G/G	A/A
C1	14,230,353	T/T	G/G	C2	13,617,381	T/T	G/G	C3	7,114,519	C/A	G/G	C3	63,484,123	C/C	T/T	C4	46,180,511	T/T	C/C	C5	21,480,742	G/G	A/A	C6	9,823,738	G/G	T/T	C7	24,687,036	G/G	A/A	C8	7,465,060	A/A	T/T	C9	1,001,641	A/A	C/C	C9	47,269,721	C/C	A/A
C1	14,775,426	A/A	C/C	C2	13,661,534	T/T	C/C	C3	7,114,545	T/T	C/C	C3	63,524,574	T/T	C/C	C4	46,180,515	G/G	A/A	C5	21,480,765	T/T	C/C	C6	9,823,751	C/C	T/T	C7	24,723,251	T/T	C/C	C8	7,534,060	G/G	C/C	C9	1,014,726	C/C	T/T	C9	47,269,730	C/C	T/T
C1	15,052,628	G/G	C/C	C2	13,661,556	T/T	G/G	C3	7,114,630	G/G	A/A	C3	63,567,265	A/A	G/G	C4	46,180,569	G/G	A/A	C5	21,480,887	C/C	T/T	C6	9,955,378	T/T	C/C	C7	24,725,099	C/C	T/T	C8	7,551,521	G/G	A/A	C9	1,028,234	G/G	T/T	C9	47,269,808	T/T	C/C
C1	15,245,188	T/T	G/G	C2	13,845,367	T/T	C/C	C3	7,131,353	C/C	T/T	C3	63,634,011	C/C	T/T	C4	46,248,851	A/A	C/C	C5	21,523,852	G/G	A/A	C6	10,211,363	A/A	G/G	C7	24,903,060	T/T	C/C	C8	7,551,537	T/T	C/C	C9	1,069,257	C/C	A/A	C9	47,269,820	A/A	T/T
C1	15,870,444	A/A	G/G	C2	13,845,388	T/T	G/G	C3	7,131,392	G/G	A/A	C3	63,634,032	T/T	A/A	C4	46,248,886	G/G	A/A	C5	21,523,916	T/T	A/A	C6	10,580,719	G/G	A/A	C7	24,953,388	A/A	T/T	C8	7,735,976	C/A	A/A	C9	1,069,276	A/A	G/G	C9	47,415,587	G/G	T/T
C1	15,965,793	G/G	A/A	C2	13,927,785	T/T	G/G	C3	7,139,422	C/C	A/A	C3	63,777,338	C/C	G/G	C4	46,267,114	T/T	A/A	C5	21,560,766	A/A	G/G	C6	10,766,560	C/G	G/G	C7	25,157,616	C/C	G/G	C8	7,736,117	A/A	T/T	C9	1,142,143	G/G	A/A	C9	47,425,375	T/T	C/C
C1	15,965,841	C/C	T/T	C2	13,928,775	G/G	C/C	C3	7,231,362	C/A	C/C	C3	63,973,327	T/T	G/G	C4	46,288,334	C/C	T/T	C5	21,680,185	G/G	A/A	C6	10,766,562	T/T	A/A	C7	25,655,252	A/A	G/G	C8	7,736,139	C/C	G/G	C9	1,180,183	G/G	A/A	C9	47,488,872	A/A	G/G
C1	15,965,875	A/A	G/G	C2	14,026,639	G/G	T/T	C3	7,246,844	A/A	C/C	C3	64,103,401	T/T	C/C	C4	46,288,347	C/C	T/T	C5	21,680,209	A/A	G/G	C6	10,766,582	G/G	C/C	C7	25,655,522	T/T	C/C	C8	7,736,201	T/T	A/A	C9	1,180,195	T/T	C/C	C9	47,488,878	C/C	

Appendix II *Cont.***Table S2: *Cont.* : Differences in Allelic variants at various chromosomes positions in parent lines; the cultivated rapid cycling DHSL150 and C07060.**

Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060												
C1	18,820,281	C/C	T/T	C2	14,949,115	C/C	A/A	C3	7,926,048	A/A	C/C	C3	64,843,534	C/C	T/T	C4	46,982,241	G/G	A/A	C5	22,689,139	G/G	A/A	C6	12,331,670	T/T	C/C	C7	28,086,085	C/C	T/T	C8	9,869,662	T/T	C/C	C9	1,693,283	G/G	A/A	C9	48,263,502	G/G	A/A
C1	18,820,313	C/C	G/G	C2	15,108,804	T/T	C/C	C3	7,926,057	C/C	G/G	C3	64,859,997	G/G	C/C	C4	46,982,244	C/C	T/T	C5	22,855,911	C/C	A/A	C6	13,052,175	G/G	T/T	C7	28,588,407	T/T	C/C	C8	9,869,664	T/T	C/C	C9	1,719,708	T/T	C/C	C9	48,282,752	C/C	G/G
C1	18,874,538	G/G	A/A	C2	15,108,848	G/G	T/T	C3	7,942,285	G/G	T/T	C3	64,860,077	G/G	A/A	C4	47,270,038	G/G	A/A	C5	22,855,930	T/T	C/C	C6	13,052,205	A/A	C/C	C7	28,847,753	G/G	C/G	C8	9,920,732	A/A	G/G	C9	1,719,788	G/G	A/A	C9	48,282,899	A/A	C/C
C1	18,874,561	T/T	C/C	C2	15,108,903	T/T	G/G	C3	7,942,296	A/A	C/C	C4	27,432	G/G	A/A	C4	47,344,949	A/A	G/G	C5	22,855,942	C/C	T/T	C6	13,237,438	C/A	A/A	C7	28,851,275	T/T	C/C	C8	10,314,816	G/G	T/T	C9	1,743,512	A/A	G/G	C9	48,516,402	G/G	A/A
C1	18,898,227	G/G	A/A	C2	15,327,513	A/A	G/G	C3	7,942,322	A/A	C/C	C4	197,276	T/T	G/G	C4	47,484,010	C/C	T/T	C5	22,855,961	G/G	A/A	C6	13,237,487	G/G	A/A	C7	28,851,281	A/A	G/G	C8	10,314,864	A/A	T/T	C9	1,798,387	C/C	T/T	C9	48,516,429	G/G	C/C
C1	18,969,935	C/C	A/C	C2	16,038,400	A/A	G/G	C3	7,942,339	A/A	G/G	C4	197,569	G/G	C/C	C4	47,484,156	A/A	T/T	C5	22,860,033	A/A	G/G	C6	13,237,507	T/T	C/C	C7	28,851,380	A/A	G/G	C8	10,533,030	A/A	G/G	C9	1,798,396	T/T	C/C	C9	48,516,432	A/A	T/T
C1	18,971,174	A/A	C/C	C2	16,338,856	T/T	C/C	C3	7,942,410	T/T	C/C	C4	504,584	T/A	A/A	C4	47,484,195	G/G	A/A	C5	22,864,351	G/G	T/T	C6	13,237,617	T/T	G/G	C7	29,179,574	G/G	A/A	C8	10,533,049	G/G	C/C	C9	1,802,779	A/A	T/T	C9	48,520,429	T/T	C/C
C1	19,085,549	A/A	T/T	C2	16,338,871	T/T	C/C	C3	7,959,361	G/G	A/A	C4	650,967	A/A	G/G	C4	47,484,268	T/T	C/C	C5	22,864,377	A/A	G/G	C6	13,502,210	G/G	A/A	C7	29,179,575	C/C	T/T	C8	10,542,362	G/G	T/T	C9	1,803,866	C/C	A/A	C9	48,520,487	G/G	C/C
C1	19,093,607	T/T	G/G	C2	16,338,917	A/A	G/G	C3	8,041,912	G/G	T/T	C4	651,022	A/A	G/G	C4	47,510,906	C/C	A/A	C5	22,871,865	C/C	T/T	C6	13,544,449	G/G	C/C	C7	29,188,333	G/G	T/T	C8	10,616,704	A/A	G/G	C9	1,876,703	A/A	G/G	C9	48,520,495	G/G	A/A
C1	19,131,832	A/A	G/G	C2	16,342,924	G/G	A/A	C3	8,151,024	T/T	C/C	C4	693,456	G/G	A/A	C4	47,631,477	A/A	G/G	C5	22,945,117	T/T	C/C	C6	14,017,162	G/G	A/A	C7	29,188,341	C/C	T/T	C8	10,855,615	T/T	G/G	C9	1,881,325	A/A	G/G	C9	48,520,538	C/C	T/T
C1	19,131,838	G/G	T/T	C2	16,345,116	C/C	T/T	C3	8,151,066	A/A	G/G	C4	693,464	A/A	T/T	C4	47,631,510	T/T	C/C	C5	22,947,489	A/A	C/C	C6	14,039,734	C/A	T/T	C7	29,188,363	G/G	T/T	C8	10,855,681	A/A	C/C	C9	1,915,714	G/G	A/A	C9	48,534,132	G/G	A/A
C1	19,146,597	A/A	G/G	C2	16,345,124	G/G	C/C	C3	8,206,956	G/G	A/A	C4	693,595	T/T	C/C	C4	47,700,560	A/A	G/G	C5	22,960,109	T/T	C/C	C6	14,039,770	T/T	G/G	C7	29,221,254	T/T	C/C	C8	10,855,692	T/T	C/C	C9	1,915,745	T/T	C/C	C9	48,595,825	T/T	C/C
C1	19,146,647	T/T	A/C	C2	16,445,606	C/C	T/T	C3	8,686,324	G/G	A/A	C4	693,623	A/A	T/T	C4	47,776,313	G/G	A/A	C5	22,972,288	A/A	G/G	C6	14,054,298	C/G	G/G	C7	29,275,116	C/C	T/T	C8	10,855,794	C/C	T/T	C9	2,099,411	T/T	C/C	C9	48,595,827	A/A	C/C
C1	19,146,676	C/C	T/T	C2	16,472,455	C/C	A/A	C3	8,693,826	A/A	C/C	C4	693,627	A/A	G/G	C4	47,778,192	A/A	G/G	C5	22,978,933	T/T	C/C	C6	14,054,302	C/G	G/G	C7	29,275,127	A/A	C/C	C8	10,936,128	T/T	C/C	C9	2,099,460	G/G	A/A	C9	48,760,563	T/T	C/C
C1	19,158,373	A/A	T/T	C2	16,610,522	A/A	T/T	C3	8,876,369	C/C	T/T	C4	694,016	G/G	C/C	C4	47,778,803	T/T	C/C	C5	22,978,968	A/A	G/G	C6	14,349,148	A/A	C/C	C7	29,275,235	A/A	G/G	C8	11,102,535	T/T	G/G	C9	2,099,559	T/T	C/C	C9	48,760,581	T/T	G/G
C1	19,186,631	T/T	G/G	C2	16,763,752	A/A	C/C	C3	8,876,372	C/C	G/A	C4	1,354,904	A/A	G/G	C4	47,779,078	G/G	T/T	C5	22,990,459	T/T	C/C	C6	14,349,215	T/T	C/C	C7	29,275,282	G/G	A/A	C8	11,141,461	G/G	A/A	C9	2,119,396	C/C	T/T	C9	48,782,476	A/A	G/G
C1	19,186,643	G/G	A/A	C2	16,851,254	C/C	G/G	C3	8,883,916	G/G	A/A	C4	1,359,488	A/A	G/G	C4	47,899,315	C/C	T/T	C5	22,990,462	C/C	T/T	C6	14,403,413	C/C	T/T	C7	29,280,478	A/A	G/G	C8	11,141,647	A/A	G/G	C9	2,126,893	A/A	T/T	C9	48,820,416	T/T	G/G
C1	19,186,668	T/A	A/A	C2	16,851,266	C/A	A/A	C3	9,317,553	T/T	G/G	C4	1,569,461	A/A	T/T	C4	47,899,420	C/C	G/G	C5	23,120,852	A/A	G/G	C6	14,437,323	C/C	T/T	C7	29,343,016	A/A	T/T	C8	11,155,615	G/G	A/A	C9	2,127,020	G/G	C/C	C9	48,857,017	C/C	T/T
C1	19,192,940	C/C	A/A	C2	16,851,472	A/A	G/G	C3	9,318,129	G/G	A/A	C4	1,621,409	A/A	T/T	C4	47,948,478	G/G	T/T	C5	23,120,862	C/C	T/T	C6	14,438,203	G/G	C/C	C7	29,382,544	A/A	C/C	C8	11,182,445	T/T	A/A	C9	2,183,903	A/A	T/T	C9	48,868,167	G/G	A/A
C1	19,225,050	C/C	A/A	C2	16,851,473	A/A	C/C	C3	9,318,132	T/T	C/C	C4	1,655,059	C/C	G/G	C4	47,948,487	T/T	G/G	C5	23,205,916	G/G	A/A	C6	14,445,232	A/A	C/C	C7	29,417,173	T/T	C/C	C8	11,476,781	T/T	G/G	C9	2,292,004	A/A	T/T	C9	48,868,213	G/G	A/A
C1	19,225,052	T/T	A/A	C2	16,853,120	A/A	C/C	C3	9,360,699	T/T	G/G	C4	1,695,981	G/G	A/A	C4	48,098,581	G/G	A/A	C5	23,273,201	G/G	T/T	C6	14,446,981	A/A	T/T	C7	29,837,871	C/C	G/G	C8	11,572,514	T/T	C/C	C9	2,304,505	G/G	C/C	C9	48,909,593	G/G	C/C
C1	19,334,165	C/C	C/C	C2	16,994,975	C/C	T/T	C3	9,386,873	G/G	T/T	C4	1,772,665	T/T	C/C	C4	48,170,826	C/C	G/G	C5	23,274,315	G/G	A/A	C6	14,446,995	A/A	C/C	C7	29,898,537	T/T	C/C	C8	11,573,225	G/G	A/A	C9	2,430,734	T/T	A/A	C9	48,909,596	T/T	G/G
C1	19,427,234	T/T	A/C	C2	16,994,993	T/T	C/C	C3	9,467,140	A/A	G/G	C4	1,772,667	G/G	C/C	C4	48,170,842	A/A	G/G	C5	23,355,201	T/T	C/C	C6	14,497,145	A/A	G/G	C7	30,272,798	A/A	G/G	C8	11,621,950	T/T	C/A	C9	2,481,085	G/G	A/A	C9	48,909,705	G/G	A/A
C1	19,496,756	T/T	C/C	C2	16,998,613	A/A	G/G	C3	9,467,222	T/T	A/A	C4	1,824,023	G/G	A/A	C4	48,170,929	T/T	G/G	C5	23,400,445	T/T	C/C	C6	14,497,159	G/G	T/T	C7	30,274,892	T/T	C/C	C8	11,820,642	T/T	C/C	C9	2,481,182	T/T	G/G	C9	48,952,408	G/G	A/A
C1	21,159,895	A/A	T/T	C2	16,998,625	G/G	C/C	C3	9,467,253	A/A	T/T	C4	1,824,065	T/T	G/G	C4	48,181,006	C/C	T/T	C5	23,438,994	T/T	C/C	C6	15,770,888	T/T	G/G	C7	30,274,913	A/A	G/G	C8	11,886,823	G/G	A/A	C9	2,491,078	T/T	C/C	C9	48,952,429	A/A	C/C
C1	21,159,898	C/C	T/T	C2	16,998,636	C/C	A/A	C3	9,527,898	C/C	T/T	C4	1,824,071	G/G	A/A	C4	48,183,914	T/T	C/C	C5	23,498,203	A/A	C/C	C6	16,822,776	T/T	A/A	C7	30,276,319	T/T	C/C	C8	11,886,847	T/T	A/A	C9	2,516,240	A/A	G/G	C9	48,952,439	C/C	T/T
C1	21,235,067	A/A	T/T	C2	17,044,040	G/G	T/T	C3	9,527,986	G/G	T/T	C4	1,824,098	C/C	T/T	C4	48,199,496	A/A	G/G	C5	23,858,245	T/T	G/G	C6	16,803,664	T/T	A/A	C7	30,363,990	A/A	G/G	C8	11,918,426	A/A	G/G	C9	2,516,311	C/C	T/T	C9	49,002,800	G/G	T/T
C1	21,235,104	A/A	G/G	C2	17,044,043	A/A	T/T	C3	9,551,448	C/C	T/T	C4	2,012,180	T/T	A/A	C4	48,200,823	A/A	G/G	C5	23,892,817	T/T	A/A	C6	16,821,537	T/T	C/C	C7	30,373,325	C/C	T/T	C8	11,958,099	A/A	G/G	C9	2,682,728	A/A	T/T	C9	49,098,183	G/G	A/A
C1	21,244,075	G/G	T/T	C2	17,076,430	A/A	C/C	C3	9,551,513	A/A	T/T	C4	2,033,285	A/A	G/G	C4	48,235,393	C/C	G/G	C5	23,953,073	C/C	G/G	C6	16,821,652	A/A	C/C	C7	30,373,330	G/G	A/A	C8	11,958,126	C/C	T/T	C9	2,712,281	G/G	A/A	C9	49,123,435	G/G	T/T
C1	21,244,096	G/G	T/T	C2	17,206,879	A/A	T/T	C3	9,551,595	C/C	T/T	C4	2,033,372	G/G	A/A	C4	48,237,288	C/C	A/A	C5	23,978,896	T/T	G/G	C6	16,828,854	C/C	C/C	C7	30,520,133	T/T	A/A	C8	11,958,134	C/C	T/T	C9	2,717,082	A/A	C/A	C9	49,123,459	T/T	C/C
C1	21,244,182	G/G	A/A	C2	17,206,892	A/A	T/T	C3	9,576,150	T/T	C/C	C4	2,033,577	C/C	G/G	C4	48,237,293	T/T	C/C	C5	23,985,832	G/G	T/T	C6	16,900,661	G/G	T/T	C7	30,520,162	C/C	T/T	C8	12,002,883	A/A	T/T	C9	2,787,348	G/G	A/A	C9	49,123,462	G/G	A/A
C1	21,244,186	G/G	T/T	C2	17,206,899	T/T	C/C	C3	9,647,191	G/G	A/A	C4	2,041,788	A/A	C/A	C4	48,295,366	T/T	C/C	C5	23,995,207	C/C	A/A	C6	17,009,572	T/T	A/A	C7	30,581,959	A/A	C/C	C8	12,007,369	A/A	T/T	C9	2,864,108	C/C	T/T	C9	49,130,826	A/A	T/T
C1	21,449,809	T/T	C/C	C2	17,262,663	C/C	T/T	C3	9,647,193	G/G	C/C	C4	2,041,898	T/T	C/C	C4	48,300,870	C/C	T/T	C5	23,995,282	C/C	T/T	C6	17,009,598	A/A	C/C	C7	30,581,962	A/A	T/T	C8	12,007,409	T/T									

Appendix II Cont.

Table S2: Cont. : Differences in Allelic variants at various chromosomes positions in parent lines; the cultivated rapid cycling DHSL150 and C07060.

Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060												
C1	21,678,746	A/A	T/T	C2	17,792,083	G/G	C/C	C3	9,899,580	T/T	C/C	C4	2,879,915	G/G	C/C	C4	48,899,984	A/A	G/G	C5	24,345,771	C/C	T/T	C6	17,681,265	T/T	C/C	C7	30,857,076	A/A	C/C	C8	12,403,114	A/A	T/T	C9	3,367,074	A/A	G/G	C9	49,230,395	T/T	G/G
C1	21,678,967	T/T	C/C	C2	18,300,245	C/C	T/T	C3	9,944,110	C/C	T/T	C4	2,879,920	T/T	C/C	C4	48,929,920	C/C	T/T	C5	24,345,776	T/T	G/G	C6	17,681,454	G/G	A/A	C7	31,022,187	G/G	T/T	C8	12,407,509	C/C	T/T	C9	3,378,507	G/G	A/A	C9	49,245,582	C/C	T/T
C1	21,680,940	C/C	T/T	C2	18,308,509	G/G	C/C	C3	9,944,122	T/T	A/A	C4	2,893,974	T/T	A/A	C4	49,061,073	A/A	G/G	C5	24,387,628	T/T	C/C	C6	17,681,563	G/G	A/A	C7	31,041,702	C/C	T/T	C8	12,423,569	G/G	A/A	C9	3,378,518	G/G	A/A	C9	49,261,829	C/C	A/A
C1	21,680,952	C/C	T/T	C2	18,308,544	T/T	C/C	C3	9,980,664	C/C	T/T	C4	2,894,531	A/A	T/T	C4	49,061,077	C/C	T/T	C5	24,387,637	C/C	G/G	C6	17,712,857	A/A	G/G	C7	31,041,835	C/C	G/G	C8	12,492,728	C/C	A/A	C9	3,389,365	G/G	A/A	C9	49,261,838	A/A	C/C
C1	21,713,593	G/G	A/A	C2	18,506,749	A/A	G/G	C3	10,325,406	C/C	T/T	C4	2,894,588	G/G	A/A	C4	49,061,103	T/T	C/C	C5	24,433,681	G/G	A/A	C6	17,808,658	A/A	T/T	C7	31,988,453	G/G	A/A	C8	12,517,097	A/A	G/G	C9	3,434,138	G/G	C/C	C9	49,377,551	C/C	T/T
C1	21,978,946	T/T	G/C	C2	18,506,750	T/T	A/A	C3	10,418,778	C/A	A/A	C4	2,946,035	C/C	A/A	C4	49,153,871	C/C	T/T	C5	24,433,797	G/G	A/A	C6	17,808,667	A/A	T/T	C7	32,462,135	G/G	T/T	C8	12,579,422	C/C	T/T	C9	3,434,171	G/G	A/A	C9	49,393,159	T/T	C/C
C1	21,978,970	A/A	C/C	C2	18,508,867	A/A	T/T	C3	10,418,779	T/T	C/C	C4	2,949,735	G/G	A/A	C4	49,153,874	A/A	G/G	C5	24,521,882	A/A	G/G	C6	17,830,129	T/T	G/G	C7	32,462,363	G/G	T/T	C8	12,612,132	G/G	A/A	C9	3,434,180	C/C	G/G	C9	49,474,336	G/G	A/A
C1	22,067,080	A/A	G/G	C2	18,509,703	G/G	A/A	C3	10,422,479	A/A	C/C	C4	3,002,878	T/T	C/C	C4	49,174,707	A/A	T/T	C5	24,540,235	A/A	G/G	C6	17,876,820	C/C	T/T	C7	32,462,405	A/A	G/G	C8	12,612,205	T/T	G/G	C9	3,434,217	C/C	T/T	C9	49,474,749	G/G	A/A
C1	22,100,238	C/C	T/T	C2	18,520,916	A/A	T/T	C3	10,449,659	A/A	T/T	C4	3,402,751	G/G	T/T	C4	49,191,927	C/C	G/G	C5	24,724,808	T/T	A/A	C6	18,259,772	T/T	A/A	C7	32,478,536	G/G	A/A	C8	12,623,655	C/C	T/T	C9	3,434,289	C/C	T/T	C9	49,474,824	A/A	G/G
C1	22,110,300	G/G	A/A	C2	18,555,285	C/C	T/T	C3	10,449,678	A/A	C/C	C4	3,402,799	A/A	G/G	C4	49,191,975	A/A	G/G	C5	25,424,750	T/T	C/C	C6	18,259,837	A/A	T/T	C7	32,622,853	T/T	C/C	C8	12,733,948	C/C	T/T	C9	3,470,658	T/T	C/C	C9	49,474,897	G/G	A/A
C1	22,160,961	G/G	C/C	C2	18,555,360	T/T	A/A	C3	10,751,224	C/C	T/T	C4	3,535,281	A/A	G/G	C4	49,191,984	T/T	G/G	C5	26,022,942	A/A	C/C	C6	18,260,801	A/A	G/G	C7	32,832,723	G/G	A/A	C8	12,736,056	G/G	A/A	C9	3,470,675	T/T	A/A	C9	49,484,618	A/A	T/T
C1	22,161,496	G/G	A/A	C2	18,800,929	G/G	T/T	C3	10,751,316	T/T	C/C	C4	3,576,714	A/A	G/G	C4	49,192,016	G/G	A/A	C5	26,129,758	G/G	T/T	C6	18,261,219	A/A	G/G	C7	32,857,129	G/G	T/T	C8	12,814,675	G/G	A/A	C9	3,479,407	G/G	A/A	C9	49,484,627	A/A	T/T
C1	22,161,520	C/C	T/T	C2	18,945,695	G/G	T/T	C3	10,751,419	A/A	G/G	C4	3,604,335	T/T	C/C	C4	49,369,446	A/A	T/T	C5	26,129,763	A/A	G/G	C6	18,261,222	A/A	G/G	C7	32,857,135	T/T	G/G	C8	12,814,692	A/A	T/T	C9	3,479,433	G/G	T/T	C9	49,484,645	A/A	T/T
C1	22,202,938	T/T	C/C	C2	18,968,363	T/T	C/C	C3	10,751,427	T/T	G/G	C4	3,611,303	T/T	C/C	C4	49,369,449	G/G	T/T	C5	26,131,510	A/A	C/C	C6	18,310,100	A/A	C/C	C7	32,857,153	T/T	C/C	C8	12,854,329	A/A	T/T	C9	3,513,461	C/C	G/G	C9	49,491,986	G/G	C/C
C1	22,249,109	G/G	T/T	C2	18,968,438	T/T	A/A	C3	10,751,493	G/C	A/A	C4	3,700,993	A/A	C/A	C4	49,369,470	A/A	G/G	C5	26,169,243	C/C	A/A	C6	18,394,650	C/C	T/T	C7	32,857,176	C/C	T/T	C8	12,940,234	T/T	G/G	C9	3,520,443	C/C	G/G	C9	49,492,787	T/T	C/C
C1	22,249,150	A/A	G/C	C2	19,703,881	G/G	A/A	C3	10,763,871	G/A	A/A	C4	3,701,009	T/T	C/C	C4	49,381,875	A/A	C/C	C5	26,169,271	T/T	G/G	C6	18,452,501	T/T	C/C	C7	32,857,276	A/A	G/G	C8	12,940,250	T/T	A/A	C9	3,633,554	T/T	C/C	C9	49,492,979	A/A	G/G
C1	22,357,509	A/A	G/G	C2	19,990,551	T/T	C/C	C3	10,885,445	G/C	C/C	C4	3,891,280	T/T	G/G	C4	49,424,307	G/G	A/A	C5	26,169,295	G/G	A/A	C6	18,502,359	A/A	G/G	C7	32,888,486	C/C	A/A	C8	12,941,028	C/A	A/A	C9	3,744,295	G/G	T/T	C9	49,494,962	C/C	G/G
C1	22,410,358	G/G	C/C	C2	20,650,374	A/A	C/C	C3	11,219,414	T/T	A/A	C4	3,891,316	A/A	G/G	C4	49,689,529	A/A	G/G	C5	26,389,529	A/A	G/G	C6	18,611,303	C/C	A/A	C7	32,888,502	A/A	G/G	C8	12,941,048	G/G	A/A	C9	3,744,357	A/A	T/T	C9	49,495,162	C/C	G/G
C1	22,410,391	T/T	C/C	C2	20,709,703	G/G	A/A	C3	11,303,616	T/T	G/G	C4	3,907,265	G/G	A/A	C4	49,692,865	T/T	G/G	C5	26,389,541	G/G	T/T	C6	18,626,671	G/G	A/A	C7	32,888,511	G/G	A/A	C8	12,941,061	C/C	T/T	C9	3,744,439	C/C	T/T	C9	49,496,079	T/T	A/A
C1	22,410,433	T/T	C/C	C2	21,228,314	A/A	G/G	C3	11,335,498	A/A	C/C	C4	3,975,777	G/G	C/C	C4	49,708,246	A/A	T/T	C5	26,391,512	T/T	C/C	C6	18,881,212	C/C	T/T	C7	32,983,191	C/A	A/A	C8	12,965,173	A/A	C/C	C9	3,757,516	C/C	T/T	C9	49,496,101	G/G	A/A
C1	22,535,615	A/A	G/C	C2	22,162,100	G/G	A/A	C3	11,379,133	G/G	A/A	C4	3,976,029	T/T	C/C	C4	49,708,256	T/T	C/C	C5	26,525,768	C/C	T/T	C6	18,881,290	A/A	G/G	C7	32,984,233	A/A	G/G	C8	12,965,198	A/A	C/C	C9	3,958,042	A/A	C/C	C9	49,550,885	T/T	C/C
C1	22,535,758	C/C	T/T	C2	22,174,901	G/G	C/C	C3	11,411,929	G/G	A/A	C4	3,981,041	T/T	A/A	C4	49,708,321	G/G	A/A	C5	26,525,878	G/G	T/T	C6	18,882,441	C/C	T/T	C7	33,143,121	G/G	A/A	C8	12,990,866	A/A	C/C	C9	4,011,984	C/C	T/T	C9	49,550,966	G/G	T/T
C1	22,607,654	G/G	A/A	C2	22,175,063	G/G	A/A	C3	11,423,492	T/T	G/G	C4	3,981,272	A/A	G/A	C4	49,710,149	C/A	T/T	C5	26,525,906	T/T	C/C	C6	18,954,396	G/G	A/A	C7	33,143,247	G/G	A/A	C8	12,995,366	T/T	C/C	C9	4,042,682	T/T	C/C	C9	49,550,987	T/T	C/C
C1	22,607,675	T/T	G/G	C2	22,175,097	T/T	C/C	C3	11,461,606	T/T	G/G	C4	4,104,108	T/T	G/A	C4	49,710,284	G/G	A/A	C5	26,525,945	G/G	A/A	C6	18,954,398	C/C	A/A	C7	33,149,054	A/A	T/T	C8	13,132,067	C/C	A/A	C9	4,165,824	G/G	A/A	C9	49,592,214	T/T	C/C
C1	22,687,644	T/T	G/G	C2	22,189,304	A/A	G/G	C3	11,650,257	T/T	C/C	C4	4,106,533	G/G	T/T	C4	49,710,304	C/A	A/A	C5	26,525,946	T/T	C/C	C6	19,128,946	T/T	C/C	C7	33,183,484	T/T	A/A	C8	14,131,801	G/G	A/A	C9	4,165,871	G/G	T/T	C9	49,592,232	A/A	G/G
C1	22,748,062	G/G	T/T	C2	22,189,317	C/C	G/G	C3	11,651,517	A/A	G/G	C4	4,106,627	G/G	A/A	C4	49,838,887	T/T	C/C	C5	26,549,517	C/T	C/C	C6	19,128,967	C/A	A/A	C7	33,183,546	G/G	C/C	C8	14,180,526	G/G	T/T	C9	4,165,878	C/C	T/T	C9	49,592,235	A/A	G/G
C1	22,795,794	A/A	G/G	C2	22,189,374	A/A	G/G	C3	11,668,326	T/T	C/C	C4	4,108,046	G/G	A/A	C4	49,856,523	A/A	T/T	C5	26,733,503	G/G	T/T	C6	19,128,970	A/A	C/C	C7	33,184,159	C/C	T/T	C8	14,312,018	A/A	G/G	C9	4,167,609	T/T	C/C	C9	49,593,815	C/C	T/T
C1	22,903,092	T/T	A/A	C2	22,247,142	C/C	T/T	C3	11,668,332	T/T	C/C	C4	4,108,058	A/A	G/G	C4	49,863,583	A/A	G/G	C5	26,779,879	A/A	G/G	C6	19,128,991	C/C	G/G	C7	33,208,616	G/G	A/A	C8	14,341,473	A/A	G/G	C9	4,175,216	A/A	T/T	C9	49,593,817	T/T	C/C
C1	24,241,050	T/T	A/A	C2	22,247,226	C/C	T/T	C3	11,668,338	G/G	A/A	C4	4,110,876	A/A	C/C	C4	49,876,182	C/C	T/T	C5	26,828,997	A/A	C/C	C6	19,129,032	A/A	C/C	C7	33,324,524	G/G	C/C	C8	14,363,100	G/G	A/A	C9	4,193,385	A/A	G/G	C9	49,593,845	C/C	T/T
C1	24,451,849	T/T	C/C	C2	22,320,236	T/T	C/C	C3	11,675,016	C/C	T/T	C4	4,131,802	A/A	G/A	C4	49,878,516	C/A	A/A	C5	26,901,430	T/T	G/G	C6	19,276,838	A/A	T/T	C7	33,324,551	C/C	T/T	C8	14,363,107	G/G	A/A	C9	4,218,183	C/C	A/A	C9	49,614,588	T/T	G/G
C1	24,493,949	T/T	G/G	C2	22,398,375	A/A	G/G	C3	11,675,107	C/C	T/T	C4	4,140,691	C/C	A/A	C4	50,040,844	G/G	C/C	C5	27,007,057	G/G	A/A	C6	19,442,683	C/C	T/T	C7	33,324,563	C/C	T/T	C8	14,410,946	C/C	T/T	C9	4,226,933	G/G	G/G	C9	49,680,324	C/C	T/T
C1	24,494,044	A/A	G/G	C2	22,403,590	T/T	C/C	C3	11,715,529	A/A	G/G	C4	4,140,824	C/A	T/T	C4	50,175,434	C/A	T/T	C5	27,333,522	T/T	A/A	C6	19,528,334	G/G	T/T	C7	33,324,611	A/A	G/G	C8	14,739,727	T/T	C/C	C9	4,273,871	C/C	G/G	C9	49,680,335	T/T	C/C
C1	24,500,753	A/A	G/G	C2	22,403,638	A/A	G/G	C3	11,771,544	T/T	G/G	C4	4,140,830	C/A	T/T	C4	50,260,181	T/T	C/C	C5	27,333,534	C/C	T/T	C6	19,528,396	G/G	A/A	C7	33,324,6														

Appendix II *Cont.***Table S2: *Cont.* : Differences in Allelic variants at various chromosomes positions in parent lines; the cultivated rapid cycling DHSL150 and C07060.**

Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060													
C1	25,497,605	T/T	G/G	C2	24,526,006	G/G	A/A	C3	13,230,665	T/T	C/C	C4	4,798,867	A/A	G/G	C4	51,195,596	A/A	G/G	C5	27,862,370	G/G	A/A	C6	21,056,309	C/C	A/A	C7	34,371,512	T/T	C/C	C8	17,142,807	T/T	A/A	C9	4,987,592	T/T	C/C	C9	50,068,595	G/G	G/G	T/T
C1	25,497,608	C/C	T/T	C2	24,526,811	T/T	A/A	C3	13,232,989	T/T	C/C	C4	4,965,352	T/T	A/A	C4	51,195,600	A/A	C/C	C5	27,911,912	G/G	A/A	C6	21,056,400	C/C	G/G	C7	34,379,271	T/T	A/A	C8	17,143,236	A/A	C/C	C9	4,999,655	G/G	A/A	C9	50,110,114	G/G	G/G	T/T
C1	25,503,912	G/G	A/A	C2	24,526,863	G/G	A/A	C3	13,463,226	G/G	A/A	C4	4,965,360	A/A	T/T	C4	51,222,103	T/T	C/C	C5	27,911,973	C/C	A/A	C6	21,216,979	A/A	C/C	C7	34,379,316	C/C	T/T	C8	17,270,614	G/G	C/C	C9	5,002,649	C/C	T/T	C9	50,110,173	C/C	A/A	A/A
C1	25,503,933	G/G	T/T	C2	24,993,054	T/T	A/A	C3	13,517,133	G/G	C/C	C4	5,108,928	T/T	A/A	C4	51,222,204	T/T	A/A	C5	27,912,007	C/C	A/A	C6	21,586,940	C/C	T/T	C7	34,381,001	G/G	A/A	C8	17,270,748	C/C	G/G	C9	5,002,716	T/T	A/A	C9	50,123,766	A/A	T/T	A/A
C1	25,542,314	T/T	G/G	C2	24,993,087	C/C	T/T	C3	13,517,279	G/G	A/A	C4	5,111,009	G/G	T/T	C4	51,222,205	T/T	A/A	C5	27,915,667	A/A	C/C	C6	21,693,876	G/G	C/C	C7	34,395,218	G/G	T/T	C8	17,274,333	A/A	G/G	C9	5,006,095	T/T	C/C	C9	50,247,213	C/C	T/T	T/T
C1	25,569,853	A/A	T/T	C2	25,005,630	T/T	C/C	C3	13,530,086	T/T	G/G	C4	5,111,014	C/C	G/G	C4	51,222,455	T/T	C/C	C5	27,915,686	G/G	T/T	C6	21,694,399	A/A	G/G	C7	34,395,224	T/T	A/A	C8	17,360,429	C/C	T/T	C9	5,043,650	A/A	G/G	C9	50,270,867	A/A	T/T	T/T
C1	25,569,872	G/G	A/A	C2	25,005,645	T/T	C/C	C3	13,530,132	G/G	C/C	C4	5,115,629	G/G	T/T	C4	51,225,463	T/T	C/C	C5	27,915,780	T/T	C/C	C6	21,694,642	G/G	A/A	C7	34,445,751	G/G	A/A	C8	17,487,192	C/C	T/T	C9	5,062,210	A/A	G/G	C9	50,305,915	T/T	C/C	A/A
C1	25,660,365	G/G	A/A	C2	25,109,023	T/T	C/C	C3	13,530,174	A/A	T/T	C4	5,115,755	C/C	A/A	C4	51,225,555	T/T	C/C	C5	27,915,840	T/T	C/C	C6	21,744,378	T/T	G/G	C7	34,445,754	G/G	A/A	C8	17,487,331	G/G	A/A	C9	5,062,220	C/C	T/T	C9	50,451,120	G/G	A/A	A/A
C1	25,675,031	A/A	C/C	C2	25,109,056	T/T	A/A	C3	13,536,179	C/C	A/A	C4	5,115,904	C/C	T/T	C4	51,225,641	G/G	A/A	C5	27,915,953	A/A	G/G	C6	21,744,427	G/G	A/A	C7	34,453,242	G/G	T/T	C8	17,521,810	C/C	G/G	C9	5,082,420	C/C	T/T	C9	50,451,124	G/G	A/A	A/A
C1	25,756,023	G/G	T/T	C2	25,264,181	C/C	A/A	C3	13,536,314	C/C	T/T	C4	5,382,109	G/G	T/T	C4	51,279,064	C/C	T/T	C5	27,958,946	C/C	T/T	C6	21,819,775	A/A	C/C	C7	34,468,205	G/G	C/C	C8	17,625,745	A/A	T/T	C9	5,083,446	T/T	G/G	C9	50,484,993	G/G	T/T	A/A
C1	25,756,047	C/C	T/T	C2	25,264,855	G/G	A/A	C3	13,537,109	G/G	A/A	C4	5,382,115	C/C	T/T	C4	51,365,287	T/T	C/C	C5	27,958,982	A/A	G/G	C6	21,819,784	T/T	G/G	C7	34,534,964	A/A	C/C	C8	17,625,817	G/G	C/C	C9	5,094,242	T/T	C/C	C9	50,485,010	G/G	A/A	A/A
C1	25,767,652	T/T	C/C	C2	25,264,876	C/C	T/T	C3	13,537,134	G/G	A/A	C4	5,405,211	C/C	T/T	C4	51,365,300	T/T	G/G	C5	27,958,995	A/A	G/G	C6	21,895,607	T/T	C/C	C7	34,583,443	G/G	A/A	C8	17,625,831	G/G	A/A	C9	5,094,248	G/G	T/T	C9	50,485,041	G/G	T/T	T/T
C1	25,927,627	C/C	T/T	C2	25,275,166	T/T	C/C	C3	13,564,086	C/C	T/T	C4	5,415,403	G/G	C/C	C4	51,419,212	T/T	C/C	C5	28,071,422	C/C	T/T	C6	21,955,320	T/T	C/C	C7	34,632,728	T/T	A/A	C8	17,813,208	C/C	G/G	C9	5,114,103	T/T	A/A	C9	50,485,076	G/G	A/A	A/A
C1	26,004,641	G/G	A/A	C2	25,461,861	G/G	A/A	C3	13,567,915	G/G	C/C	C4	5,615,139	T/T	C/C	C4	51,419,237	T/T	C/C	C5	28,091,643	G/G	A/A	C6	21,955,343	A/A	G/G	C7	34,697,753	T/T	G/G	C8	17,856,007	T/T	G/G	C9	5,114,309	T/T	G/G	C9	50,485,106	G/G	A/A	A/A
C1	26,022,328	C/C	G/G	C2	25,567,114	A/A	G/G	C3	13,761,592	C/C	G/G	C4	5,648,831	G/G	A/A	C4	51,533,624	T/T	A/A	C5	28,114,307	G/G	C/C	C6	21,970,583	A/A	C/C	C7	34,697,876	T/T	C/C	C8	18,338,432	G/G	T/T	C9	5,134,526	T/T	A/A	C9	50,500,333	C/C	T/T	T/T
C1	26,067,250	G/G	T/T	C2	25,581,142	C/C	A/A	C3	13,761,644	G/G	T/T	C4	5,648,834	A/A	C/C	C4	51,533,645	A/A	G/G	C5	28,165,395	T/T	G/G	C6	21,970,589	A/A	C/C	C7	34,719,340	C/C	G/G	C8	18,696,447	C/C	G/G	C9	5,134,530	A/A	G/G	C9	50,500,370	T/T	A/A	A/A
C1	26,151,205	T/T	C/C	C2	27,390,255	T/T	C/C	C3	13,761,668	G/G	A/A	C4	5,754,329	C/C	T/T	C4	51,533,672	C/C	A/A	C5	28,210,962	G/G	C/C	C6	21,970,597	A/A	C/C	C7	34,740,550	C/C	T/T	C8	18,751,403	T/T	C/C	C9	5,134,581	G/G	C/C	C9	50,500,381	G/G	A/A	A/A
C1	26,153,682	T/T	A/A	C2	27,562,361	G/G	T/T	C3	13,762,449	A/A	G/G	C4	6,042,715	T/T	C/C	C4	51,540,314	A/A	T/T	C5	28,210,990	C/C	A/A	C6	22,392,657	C/C	G/G	C7	34,747,578	C/C	T/T	C8	18,751,413	C/C	A/A	C9	5,370,151	C/C	A/A	C9	50,559,815	C/C	A/A	A/A
C1	26,238,845	T/T	G/G	C2	27,696,466	C/C	T/T	C3	13,768,044	A/A	G/G	C4	6,042,721	G/G	T/T	C4	51,564,157	C/C	A/A	C5	28,212,834	G/G	A/A	C6	22,399,168	G/G	C/C	C7	34,747,593	G/G	A/A	C8	19,026,574	T/T	C/C	C9	5,370,182	C/C	T/T	C9	50,593,275	G/G	A/A	A/A
C1	26,268,633	A/A	G/G	C2	27,698,824	A/A	A/A	C3	13,768,158	C/C	A/A	C4	6,044,438	T/T	A/A	C4	51,600,968	C/C	C/C	C5	28,215,654	G/G	A/A	C6	22,399,201	T/T	C/C	C7	34,748,595	C/C	T/T	C8	19,026,584	A/A	G/G	C9	5,372,037	G/G	A/A	C9	50,593,368	T/T	A/A	C/A
C1	26,310,705	C/C	T/T	C2	27,956,167	G/G	T/T	C3	13,853,766	C/C	A/A	C4	6,152,836	C/C	T/T	C4	51,601,049	C/C	A/A	C5	28,926,939	C/C	T/T	C6	22,434,009	A/A	G/G	C7	34,769,375	T/T	A/A	C8	19,026,590	A/A	G/G	C9	5,372,111	A/A	G/G	C9	50,613,214	A/A	G/G	A/A
C1	26,310,725	C/A	A/A	C2	27,962,371	T/T	A/A	C3	13,993,737	T/T	A/A	C4	6,152,851	A/A	C/C	C4	51,601,061	A/A	G/G	C5	28,949,780	A/A	A/A	C6	22,434,161	C/C	T/T	C7	34,769,408	T/T	A/A	C8	19,031,912	T/T	A/A	C9	5,372,133	T/T	C/C	C9	50,613,215	G/G	A/A	A/A
C1	26,310,747	T/T	A/A	C2	27,962,507	A/A	G/G	C3	13,993,832	C/C	T/T	C4	6,194,375	C/C	T/T	C4	51,699,903	T/T	G/G	C5	28,949,884	G/G	A/A	C6	22,434,185	T/T	C/C	C7	34,773,029	A/A	C/C	C8	19,034,509	A/A	G/G	C9	5,372,701	C/C	T/T	C9	50,613,229	T/T	A/A	C/A
C1	26,487,947	A/A	G/G	C2	27,963,636	A/A	T/T	C3	13,994,061	A/A	T/T	C4	6,194,433	C/C	T/T	C4	51,699,975	C/C	T/T	C5	28,949,894	A/A	G/G	C6	22,434,219	T/T	C/C	C7	34,828,316	T/T	C/C	C8	19,034,515	C/C	T/T	C9	5,378,898	A/A	G/G	C9	50,724,328	T/T	A/A	A/A
C1	26,501,464	T/T	C/C	C2	28,272,017	T/T	C/C	C3	14,069,280	T/T	A/A	C4	6,194,476	C/C	A/A	C4	51,700,014	A/A	T/T	C5	28,949,915	T/T	C/C	C6	22,434,233	G/G	A/A	C7	34,828,376	A/A	G/G	C8	19,041,898	T/T	C/C	C9	5,379,792	T/T	A/A	C9	50,780,119	C/C	T/T	A/A
C1	26,590,091	T/T	A/A	C2	28,413,869	G/G	A/A	C3	14,069,281	T/T	A/A	C4	6,194,519	G/G	A/A	C4	51,700,029	A/A	G/G	C5	28,953,191	T/T	C/C	C6	22,441,437	A/A	C/C	C7	35,135,589	T/T	C/C	C8	19,061,669	C/C	T/T	C9	5,396,316	T/T	G/G	C9	50,780,284	T/T	G/G	A/A
C1	26,590,223	T/T	G/G	C2	28,463,859	T/T	A/A	C3	14,083,611	G/G	A/A	C4	6,203,704	C/C	T/T	C4	51,700,182	C/C	T/T	C5	28,953,198	G/G	T/T	C6	22,441,446	C/C	T/T	C7	35,144,643	T/T	A/A	C8	19,165,813	T/T	C/C	C9	5,396,427	A/A	C/C	C9	50,780,293	G/G	C/C	A/A
C1	26,620,883	T/T	C/C	C2	28,474,758	A/A	G/G	C3	14,178,085	A/A	C/C	C4	6,243,688	C/C	G/G	C4	51,700,203	C/C	T/T	C5	28,953,236	C/C	T/T	C6	23,256,321	A/A	T/T	C7	35,144,708	C/C	T/T	C8	19,165,865	A/A	T/T	C9	5,396,436	A/A	G/G	C9	50,780,296	G/G	A/A	A/A
C1	26,691,287	A/A	G/G	C2	28,515,552	G/G	A/A	C3	14,178,867	G/G	A/A	C4	6,295,251	A/A	C/C	C4	51,700,233	C/C	T/T	C5	28,953,250	A/A	G/G	C6	23,268,161	T/T	A/A	C7	35,178,966	C/C	T/T	C8	19,305,965	C/C	T/T	C9	5,396,451	G/G	A/A	C9	50,781,865	A/A	G/G	A/A
C1	26,706,491	T/T	G/G	C2	28,515,571	A/A	C/C	C3	14,437,818	G/G	C/C	C4	6,351,117	G/G	A/A	C4	51,700,248	G/G	A/A	C5	28,953,259	A/A	G/G	C6	23,299,877	C/C	A/A	C7	35,205,845	T/T	C/C	C8	19,305,981	A/A	G/G	C9	5,398,059	A/A	T/T	C9	50,793,146	C/C	G/G	A/A
C1	26,777,731	G/G	C/C	C2	28,633,140	T/T	C/C	C3	14,572,865	G/G	A/A	C4	6,535,058	A/A	G/A	C4	51,700,293	G/A	A/A	C5	29,003,658	T/T	A/A	C6	23,301,501	A/A	T/T	C7	35,451,426	C/C	T/T	C8	20,231,936	A/A	G/G	C9	5,454,300	T/T	C/C	C9	50,793,261	A/A	T/T	A/A
C1	26,877,743	A/A	C/C	C2	28,633,310	G/G	T/T	C3	15,215,178	T/T	G/G	C4	6,571,438	A/A	C/C	C4	51,700,560	A/A	G/G	C5	29,206,410	T/T	A/A	C6	23,301,608	C/C	T/T	C7	35,451,427	A/A	C/C	C8	20,244,281	A/A	G/G	C9	5,455,039	G/G	A/A	C9	50,813,656	A/A	G/G	A/A
C1	27,053,962	C/C	T/T	C2																																								

Appendix II Cont.

Table S2: Cont. : Differences in Allelic variants at various chromosomes positions in parent lines; the cultivated rapid cycling DHSL150 and C07060.

Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060												
C1	29,268,506	T/T	G/G	C2	29,582,860	T/T	C/C	C3	16,625,307	A/A	C/C	C4	7,354,725	A/A	C/C	C4	52,292,154	G/G	T/T	C5	29,685,817	A/A	G/G	C6	23,888,254	T/T	G/G	C7	35,894,703	G/G	T/T	C8	21,090,948	G/G	T/T	C9	6,016,509	G/G	A/A	C9	51,323,388	T/T	G/G
C1	29,289,137	G/G	A/A	C2	29,594,110	G/G	C/C	C3	16,625,308	T/T	G/G	C4	7,354,726	T/T	C/C	C4	52,292,252	A/A	G/G	C5	29,763,811	A/A	C/C	C6	23,889,209	A/A	T/T	C7	35,906,207	G/G	C/C	C8	21,131,404	A/A	C/C	C9	6,016,652	C/C	T/T	C9	51,374,430	T/T	G/G
C1	29,289,310	G/G	T/T	C2	29,679,960	C/C	T/T	C3	16,637,882	A/A	G/G	C4	7,356,081	T/T	C/C	C4	52,434,009	G/G	A/A	C5	30,134,341	C/C	T/T	C6	23,889,251	A/A	C/C	C7	35,906,268	T/T	G/G	C8	21,133,174	G/G	A/A	C9	6,016,667	T/T	C/C	C9	51,374,673	G/G	A/A
C1	29,289,318	G/G	T/T	C2	29,761,345	G/G	A/A	C3	16,637,904	A/A	G/G	C4	7,358,765	C/G	G/G	C4	52,653,014	G/G	A/A	C5	30,473,559	A/A	G/G	C6	24,037,314	C/C	T/T	C7	35,920,760	A/A	G/G	C8	21,143,071	C/C	A/A	C9	6,017,706	T/T	G/G	C9	51,374,757	C/C	T/T
C1	29,537,709	G/G	A/A	C2	29,761,375	T/T	G/G	C3	16,717,778	G/G	A/A	C4	7,382,421	A/A	C/C	C4	52,653,989	T/T	G/G	C5	30,547,779	A/A	C/C	C6	24,037,363	T/T	C/C	C7	35,932,239	C/C	T/T	C8	21,228,496	T/T	C/C	C9	6,017,723	G/G	A/A	C9	51,394,161	G/G	T/T
C1	30,096,261	G/G	A/A	C2	29,918,525	C/T	T/T	C3	16,767,635	A/A	C/C	C4	7,382,522	G/G	C/G	C4	52,680,714	A/A	G/G	C5	30,857,435	C/A	C/C	C6	24,037,401	G/G	A/A	C7	35,932,269	A/A	G/G	C8	21,329,260	A/A	T/T	C9	6,017,757	A/A	T/T	C9	51,425,250	A/A	G/G
C1	30,531,595	C/A	A/A	C2	29,918,543	T/T	A/A	C3	17,006,470	T/T	C/C	C4	7,382,556	T/T	C/C	C4	52,761,978	A/A	G/G	C5	30,867,821	C/A	A/A	C6	24,164,694	G/G	T/T	C7	35,934,293	A/A	G/G	C8	21,370,893	G/G	A/A	C9	6,081,633	A/A	G/G	C9	51,429,048	T/T	C/C
C1	30,531,608	C/C	A/A	C2	29,918,544	A/A	T/T	C3	17,006,492	G/G	A/A	C4	7,540,399	T/T	C/C	C4	52,763,880	G/G	C/C	C5	30,884,019	G/A	A/A	C6	24,224,690	A/A	G/G	C7	35,935,552	T/T	C/C	C8	21,373,840	A/A	T/T	C9	6,081,652	G/G	A/A	C9	51,438,178	G/G	A/A
C1	30,796,171	G/G	C/C	C2	29,920,010	G/G	A/A	C3	17,150,609	G/G	A/A	C4	7,884,905	C/C	T/T	C4	52,883,168	T/T	C/C	C5	30,932,277	G/G	T/T	C6	24,224,777	C/G	G/G	C7	35,937,940	G/G	A/A	C8	21,374,031	A/A	T/T	C9	6,279,923	A/A	T/T	C9	51,459,288	G/G	T/T
C1	30,918,194	G/G	A/A	C2	29,957,621	C/A	A/A	C3	17,150,642	A/A	G/G	C4	7,884,922	T/T	C/C	C4	52,892,337	T/T	C/C	C5	30,932,354	A/A	T/T	C6	24,240,121	T/T	A/A	C7	35,938,392	G/G	T/T	C8	21,439,231	A/A	G/G	C9	6,280,076	T/T	C/C	C9	51,459,292	A/A	T/T
C1	30,918,202	C/C	A/A	C2	29,957,770	A/A	C/C	C3	17,169,189	A/A	C/C	C4	7,884,937	G/G	A/A	C4	52,892,436	G/G	C/C	C5	30,946,185	G/A	A/A	C6	24,290,966	C/C	T/T	C7	35,938,413	G/G	A/A	C8	21,484,806	G/G	C/C	C9	6,280,082	T/T	A/A	C9	51,470,109	A/A	G/G
C1	30,918,232	T/T	C/C	C2	29,986,937	C/C	A/A	C3	17,238,051	C/G	G/A	C4	7,885,067	A/A	G/G	C4	52,905,413	G/G	A/A	C5	30,946,206	C/A	A/A	C6	24,347,749	C/A	A/A	C7	35,938,422	G/G	A/A	C8	21,484,873	C/G	G/G	C9	6,280,513	G/G	A/A	C9	51,470,333	A/A	G/G
C1	31,556,646	T/T	C/C	C2	29,986,965	C/C	G/G	C3	17,401,219	G/G	C/G	C4	7,943,236	A/A	A/A	C4	52,905,431	C/C	A/A	C5	30,946,238	T/T	C/C	C6	24,347,765	A/A	G/G	C7	35,951,559	G/G	A/A	C8	21,610,152	C/C	T/C	C9	6,286,681	A/A	G/A	C9	51,471,084	C/C	T/T
C1	31,885,119	G/G	T/T	C2	30,152,341	T/T	C/C	C3	17,440,784	T/T	C/C	C4	7,983,977	C/A	T/T	C4	52,905,440	G/G	T/T	C5	30,946,405	C/A	T/T	C6	24,347,778	T/T	G/G	C7	35,951,572	G/G	A/A	C8	21,778,000	T/T	C/C	C9	6,333,155	A/A	G/G	C9	51,553,184	G/G	A/A
C1	32,029,329	C/G	T/T	C2	30,169,855	C/T	T/T	C3	17,796,642	T/T	G/G	C4	7,988,787	C/A	T/T	C4	52,985,744	A/A	T/T	C5	31,033,763	T/A	T/A	C6	24,522,109	A/A	G/G	C7	35,962,026	G/A	A/A	C8	21,778,036	C/C	T/T	C9	6,364,146	G/A	A/A	C9	51,626,428	A/A	G/G
C1	32,054,268	T/T	C/C	C2	30,282,279	A/A	G/G	C3	17,796,653	A/A	C/C	C4	8,086,720	T/T	A/A	C4	53,001,634	T/T	A/A	C5	31,031,772	G/A	C/A	C6	24,536,414	G/G	T/T	C7	35,968,982	C/C	A/A	C8	21,885,900	G/A	A/A	C9	6,408,102	T/T	C/C	C9	51,685,740	C/C	T/T
C1	32,054,642	A/A	C/C	C2	30,349,372	G/G	A/A	C3	17,796,731	C/C	A/A	C4	8,242,346	T/T	G/G	C4	53,004,465	G/G	A/A	C5	31,140,538	C/C	G/G	C6	24,592,981	G/G	A/A	C7	36,054,962	G/G	C/C	C8	21,885,636	G/A	A/A	C9	6,488,707	G/A	C/C	C9	51,685,764	T/T	C/C
C1	32,055,912	A/A	G/G	C2	30,349,414	G/G	A/A	C3	17,796,765	G/A	A/A	C4	8,291,052	T/T	G/G	C4	53,125,729	C/T	T/T	C5	31,454,732	G/A	T/T	C6	24,642,631	G/G	T/T	C7	36,088,388	T/T	A/A	C8	21,885,663	G/G	C/C	C9	6,488,744	C/A	A/A	C9	51,691,635	G/G	A/A
C1	32,120,843	T/T	C/C	C2	30,762,412	G/G	A/A	C3	17,806,820	A/A	T/T	C4	8,291,067	G/A	A/A	C4	53,125,756	G/G	T/T	C5	31,454,762	A/A	G/G	C6	24,647,019	G/G	A/A	C7	36,098,399	C/T	A/A	C8	21,913,863	G/G	A/A	C9	6,507,170	G/G	A/A	C9	51,691,638	A/A	C/C
C1	32,135,987	T/T	C/C	C2	30,762,426	G/G	A/A	C3	17,898,850	G/A	A/A	C4	8,291,090	G/A	A/A	C4	53,125,768	T/T	C/C	C5	31,454,780	A/A	G/G	C6	24,704,545	T/T	C/T	C7	36,162,429	C/T	T/T	C8	21,913,920	G/G	A/A	C9	6,507,173	G/G	A/A	C9	51,695,531	T/T	C/C
C1	32,160,094	C/C	G/G	C2	30,762,495	T/T	C/C	C3	17,913,352	G/G	T/A	C4	8,299,657	A/A	C/C	C4	53,126,701	T/T	G/G	C5	31,454,823	T/T	C/C	C6	24,726,283	G/G	A/A	C7	36,162,459	A/A	G/G	C8	21,914,092	A/A	G/G	C9	6,509,147	A/A	G/G	C9	51,695,537	T/T	C/C
C1	32,165,513	G/G	C/C	C2	30,783,992	C/C	A/A	C3	17,930,155	C/C	T/T	C4	8,299,688	A/A	G/G	C4	53,127,274	T/T	C/C	C5	31,658,458	A/A	G/G	C6	24,791,469	G/G	A/A	C7	36,162,485	A/A	G/G	C8	22,443,110	A/A	G/G	C9	6,560,437	G/G	A/A	C9	51,695,651	T/T	C/C
C1	32,227,656	A/A	C/C	C2	30,783,993	A/A	C/C	C3	17,994,756	C/G	G/A	C4	8,299,693	A/A	C/C	C4	53,132,309	G/G	A/A	C5	31,658,459	T/T	G/G	C6	24,818,007	C/C	A/A	C7	36,169,156	A/A	G/G	C8	22,452,340	T/T	A/A	C9	6,560,497	G/G	T/T	C9	51,715,857	C/C	T/T
C1	32,752,455	A/A	C/C	C2	31,230,130	T/T	C/C	C3	18,149,471	C/C	T/T	C4	8,301,453	C/A	A/A	C4	53,132,348	C/C	G/G	C5	31,746,545	G/G	C/C	C6	24,818,026	C/C	T/T	C7	36,169,203	A/A	G/G	C8	22,452,409	T/T	C/C	C9	6,590,800	A/A	T/T	C9	51,791,042	A/A	G/G
C1	32,757,960	G/G	T/T	C2	31,230,162	G/G	A/A	C3	18,431,373	A/A	G/A	C4	8,344,896	A/A	G/G	C4	53,170,432	A/A	T/T	C5	31,837,703	T/T	C/C	C6	24,953,272	A/A	T/T	C7	36,239,137	A/A	G/G	C8	22,452,435	T/T	C/C	C9	6,590,804	C/C	T/T	C9	51,791,308	G/G	T/T
C1	33,081,235	A/A	C/C	C2	31,230,169	G/G	A/A	C3	18,458,392	G/A	A/A	C4	8,465,275	A/A	G/G	C4	53,180,631	A/A	G/G	C5	31,837,781	A/A	C/C	C6	24,956,494	C/C	T/T	C7	36,243,051	A/A	G/G	C8	22,462,622	G/G	A/A	C9	6,647,958	A/A	T/T	C9	51,807,336	A/A	T/T
C1	33,143,060	C/C	G/G	C2	31,230,250	G/G	C/C	C3	18,488,010	C/C	T/T	C4	8,472,559	C/A	A/A	C4	53,180,636	T/T	A/A	C5	31,837,804	G/G	C/C	C6	25,116,608	G/G	T/T	C7	36,243,078	C/C	G/G	C8	22,462,644	A/A	G/G	C9	6,659,357	T/T	C/C	C9	51,807,423	A/A	C/C
C1	33,157,769	T/T	G/G	C2	31,282,994	C/A	A/A	C3	18,488,022	C/C	T/T	C4	8,472,695	A/A	T/T	C4	53,180,650	G/G	A/A	C5	31,985,460	C/A	A/A	C6	25,116,947	A/A	G/G	C7	36,243,085	G/G	C/C	C8	22,722,139	G/G	A/A	C9	6,659,375	C/G	G/G	C9	51,877,611	C/C	C/C
C1	33,366,353	T/T	G/G	C2	31,383,328	T/T	C/C	C3	18,527,316	G/G	A/A	C4	8,472,745	G/A	A/A	C4	53,318,446	G/G	T/T	C5	31,994,510	G/G	T/T	C6	25,117,751	T/T	C/C	C7	36,243,154	G/G	A/A	C8	22,755,757	C/C	T/T	C9	6,659,489	T/T	G/G	C9	51,981,299	C/C	T/T
C1	33,399,147	T/T	A/A	C2	31,387,481	C/A	A/A	C3	18,565,765	C/G	A/A	C4	8,473,732	T/T	A/A	C4	53,318,544	A/A	G/G	C5	32,094,554	G/G	T/T	C6	25,297,776	T/T	G/G	C7	36,349,860	C/C	A/A	C8	23,020,908	G/G	T/T	C9	6,684,758	C/C	G/G	C9	52,004,464	A/A	C/C
C1	33,433,767	G/G	A/A	C2	31,412,382	A/A	T/T	C3	18,728,937	C/G	G/G	C4	8,473,816	A/A	G/A	C4	53,381,179	G/G	A/A	C5	32,094,556	G/G	C/C	C6	25,297,982	G/G	A/A	C7	36,349,865	C/C	T/T	C8	23,396,601	C/A	A/A	C9	6,684,818	T/T	A/A	C9	52,023,736	T/T	C/C
C1	33,433,904	G/G	T/T	C2	31,412,485	C/A	T/T	C3	18,737,699	C/G	G/G	C4	8,473,828	G/A	A/A	C4	53,381,189	C/C	A/A	C5	32,127,255	T/T	C/C	C6	25,421,457	G/G	A/A	C7	36,350,038	G/G	A/A	C8	23,396,614	C/C	T/T	C9	6,684,826	T/T	A/A	C9	52,023,739	A/A	G/G
C1	33,480,843	T/T	C/C	C2	31,605,965	C/C	T/T	C3	18,737,817	C/C	G/G	C4	8,528,513	C/C	T/T	C4	53,381,194	G/G	T/T	C5	32,127,288	A/A	G/G	C6	25,488,519	C/C	G/G	C7	36,3														

Appendix II Cont.

Table S2: Cont. : Differences in Allelic variants at various chromosomes positions in parent lines; the cultivated rapid cycling DHSL150 and C07060.

Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060												
C1	35,049,227	T/T	C/C	C2	33,061,848	T/T	C/C	C3	20,311,462	A/A	G/G	C4	9,324,808	C/C	T/T	C5	68,033	T/T	C/C	C5	33,134,825	C/C	A/A	C6	25,997,579	A/A	G/G	C7	37,144,840	T/T	C/C	C8	24,794,541	C/C	G/G	C9	7,582,988	A/A	G/G	C9	52,838,300	G/G	A/A
C1	35,110,638	G/G	A/A	C2	33,061,868	T/T	C/C	C3	20,311,471	A/A	G/G	C4	9,324,881	A/A	C/C	C5	85,667	G/G	A/A	C5	33,310,571	T/T	C/C	C6	25,997,596	T/T	A/A	C7	37,144,845	A/A	C/C	C8	24,938,411	C/C	T/T	C9	7,583,072	T/T	C/C	C9	52,838,306	G/G	A/A
C1	35,110,645	T/T	C/C	C2	33,158,192	G/G	T/T	C3	20,312,068	C/C	G/G	C4	9,336,044	T/T	A/A	C5	85,755	G/G	A/A	C5	33,452,993	C/C	T/T	C6	26,047,238	G/G	A/A	C7	37,146,652	A/A	G/G	C8	25,233,698	T/T	C/C	C9	7,583,080	A/A	T/T	C9	52,860,350	A/A	T/T
C1	35,110,657	T/T	G/G	C2	33,226,552	T/T	A/A	C3	20,312,102	G/G	T/T	C4	9,336,611	C/C	T/T	C5	85,759	G/G	C/C	C5	33,477,955	C/C	G/G	C6	26,272,672	T/T	G/G	C7	37,146,703	A/A	G/G	C8	25,233,728	C/C	A/A	C9	7,584,623	C/C	G/G	C9	52,908,900	T/T	C/C
C1	35,110,838	T/T	A/A	C2	33,226,588	G/G	A/A	C3	20,377,867	A/A	G/G	C4	9,480,497	C/C	A/A	C5	85,769	A/A	G/G	C5	33,477,980	T/T	C/C	C6	26,273,409	C/C	T/T	C7	37,146,743	C/C	A/A	C8	25,233,824	C/C	T/T	C9	7,585,554	C/C	T/T	C9	52,908,918	T/T	C/C
C1	35,201,996	C/C	T/T	C2	33,283,544	G/G	T/T	C3	20,377,879	A/A	C/C	C4	9,597,105	G/G	A/A	C5	100,224	T/T	G/G	C5	34,140,466	A/A	T/T	C6	26,461,872	G/G	A/A	C7	37,146,748	A/A	G/G	C8	25,289,229	G/G	A/A	C9	7,591,771	A/A	G/G	C9	52,908,951	T/T	C/C
C1	35,555,405	A/A	T/T	C2	33,359,248	T/T	G/G	C3	20,425,442	T/T	C/C	C4	9,597,112	C/C	T/T	C5	364,936	T/T	A/A	C5	34,140,566	T/T	C/C	C6	26,461,940	A/A	G/G	C7	37,226,715	T/T	A/A	C8	25,312,339	T/T	G/G	C9	7,591,939	T/T	C/C	C9	52,908,966	C/C	T/T
C1	35,555,424	G/G	A/A	C2	33,788,837	C/C	A/A	C3	20,492,512	A/A	T/T	C4	9,615,981	G/G	C/C	C5	408,243	A/A	T/T	C5	34,140,571	C/C	T/T	C6	26,518,611	C/C	T/T	C7	37,287,731	T/T	C/C	C8	25,312,801	G/G	A/A	C9	7,609,136	T/T	C/C	C9	52,908,983	A/A	T/T
C1	35,555,454	T/T	G/G	C2	33,943,714	G/G	A/A	C3	20,492,593	G/G	A/A	C4	9,616,238	C/C	T/T	C5	409,891	C/C	T/T	C5	34,142,055	G/G	T/T	C6	26,600,879	G/G	A/A	C7	37,288,313	C/C	T/T	C8	25,330,405	G/G	C/C	C9	7,664,652	G/G	A/A	C9	52,908,984	G/G	T/T
C1	35,575,628	T/T	C/C	C2	33,943,736	A/A	G/G	C3	20,492,608	C/C	A/A	C4	9,778,955	C/C	A/A	C5	409,902	G/G	A/A	C5	34,322,691	C/C	A/A	C6	26,633,769	G/G	A/A	C7	37,300,411	C/C	T/T	C8	25,451,545	G/G	T/T	C9	7,664,654	A/A	T/T	C9	52,909,074	T/T	G/G
C1	35,575,655	G/G	A/A	C2	34,034,115	T/T	G/G	C3	20,868,550	A/A	C/C	C4	9,779,084	C/C	G/G	C5	420,217	T/T	C/C	C5	34,342,587	C/C	G/G	C6	26,633,849	T/T	C/C	C7	37,304,932	C/C	T/T	C8	25,451,835	T/T	C/C	C9	7,670,794	A/A	G/G	C9	52,940,958	T/T	C/C
C1	35,634,901	C/C	A/A	C2	34,095,984	A/A	G/G	C3	20,904,015	A/A	T/T	C4	9,781,521	C/C	T/T	C5	420,241	T/T	C/C	C5	34,343,683	G/G	A/A	C6	26,633,947	C/C	G/G	C7	37,304,953	A/A	C/C	C8	25,555,504	C/C	T/T	C9	7,674,555	G/G	A/A	C9	52,960,568	A/A	T/T
C1	35,634,922	G/G	A/A	C2	34,096,028	T/T	A/A	C3	20,904,046	G/G	A/A	C4	9,781,579	T/T	C/C	C5	450,018	C/C	T/T	C5	34,343,686	A/A	T/T	C6	26,698,190	G/G	A/A	C7	37,305,069	C/C	T/T	C8	25,599,536	T/T	G/G	C9	7,674,966	G/G	T/T	C9	52,960,592	T/T	C/C
C1	35,635,879	T/T	C/C	C2	34,105,053	G/G	A/A	C3	20,904,047	A/A	C/C	C4	9,860,567	A/A	G/G	C5	542,208	G/G	A/A	C5	34,343,719	T/T	C/C	C6	26,698,822	G/G	C/C	C7	37,305,114	A/A	G/G	C8	25,711,590	A/A	G/G	C9	7,689,717	G/G	A/A	C9	53,045,961	A/A	G/G
C1	35,646,366	A/A	G/G	C2	34,105,063	A/A	G/G	C3	20,906,623	G/G	A/A	C4	9,860,584	A/A	G/G	C5	568,202	A/A	C/C	C5	34,347,467	G/G	A/A	C6	26,698,828	C/C	A/A	C7	37,326,788	G/G	T/T	C8	25,767,638	A/A	C/C	C9	7,689,723	C/C	G/G	C9	53,045,970	G/G	A/A
C1	35,710,969	C/C	T/T	C2	34,119,821	A/A	T/T	C3	20,910,535	C/C	T/T	C4	9,860,615	T/T	C/C	C5	569,102	T/T	C/C	C5	34,347,504	C/C	A/A	C6	26,698,871	C/C	A/A	C7	37,326,836	A/A	G/G	C8	26,073,137	A/A	C/C	C9	7,703,279	C/C	T/T	C9	53,045,991	G/G	A/A
C1	35,711,142	A/A	C/C	C2	34,185,295	G/G	A/A	C3	20,994,501	C/G	A/A	C4	9,878,011	G/G	A/A	C5	569,116	A/A	T/T	C5	34,414,944	T/T	A/A	C6	26,698,881	A/A	G/G	C7	37,385,582	A/A	G/G	C8	26,243,162	C/C	T/T	C9	7,757,987	G/G	A/A	C9	53,046,036	T/T	A/A
C1	35,711,145	T/T	G/G	C2	34,220,848	A/A	G/G	C3	21,058,442	C/C	A/A	C4	9,878,022	G/G	A/A	C5	593,768	A/A	G/G	C5	34,414,949	G/A	A/A	C6	26,978,661	G/G	A/A	C7	37,406,726	A/A	G/G	C8	26,246,090	T/T	G/G	C9	7,853,783	C/C	T/T	C9	53,053,948	A/A	G/G
C1	35,760,400	G/G	A/A	C2	34,451,245	A/A	G/G	C3	21,079,247	C/C	T/T	C4	10,189,060	G/G	C/C	C5	594,205	T/T	C/C	C5	34,414,980	A/A	T/T	C6	27,022,624	T/T	G/G	C7	37,562,861	G/G	A/A	C8	26,289,893	G/G	A/A	C9	7,858,095	T/T	G/G	C9	53,053,963	A/A	T/T
C1	35,764,064	G/G	T/T	C2	34,451,305	T/T	A/A	C3	21,146,811	A/A	T/T	C4	10,189,070	C/G	G/G	C5	595,102	C/C	T/T	C5	34,454,335	A/A	T/T	C6	27,078,828	G/G	C/C	C7	37,562,885	T/T	A/A	C8	26,611,539	T/T	C/C	C9	7,895,462	A/A	G/G	C9	53,053,975	A/A	T/T
C1	35,838,391	G/G	A/A	C2	34,648,759	C/C	T/T	C3	21,222,932	G/G	A/A	C4	10,222,423	C/C	G/G	C5	595,106	T/T	C/C	C5	34,459,111	G/A	A/A	C6	27,081,549	C/C	A/A	C7	37,562,936	T/T	A/A	C8	26,692,118	G/G	C/C	C9	7,896,617	C/C	T/T	C9	53,062,087	C/C	T/T
C1	36,354,173	C/C	A/A	C2	34,704,701	T/T	A/A	C3	21,226,604	G/G	A/A	C4	10,354,410	A/A	C/C	C5	651,626	G/G	T/T	C5	34,470,832	G/G	T/T	C6	27,085,881	G/G	A/A	C7	37,578,777	C/C	A/A	C8	26,734,647	C/C	T/T	C9	8,327,124	A/A	T/T	C9	53,261,214	G/G	A/A
C1	36,354,272	C/C	C/T	C2	34,704,703	T/T	C/C	C3	21,226,862	C/C	A/A	C4	10,355,614	G/A	A/A	C5	651,716	G/G	C/C	C5	34,470,860	A/A	T/T	C6	27,091,747	A/A	G/G	C7	37,578,921	T/T	C/C	C8	26,792,610	G/G	C/C	C9	8,413,272	G/G	C/C	C9	53,261,312	T/T	C/C
C1	36,526,324	C/C	T/T	C2	35,085,506	C/C	G/G	C3	21,232,913	A/A	G/G	C4	10,355,640	C/C	T/T	C5	651,788	G/G	A/A	C5	34,553,495	C/C	G/G	C6	27,103,657	C/C	T/T	C7	37,578,951	A/A	C/C	C8	26,792,653	A/A	T/T	C9	8,413,723	C/C	T/T	C9	53,266,711	A/A	C/C
C1	36,552,104	G/G	A/A	C2	35,105,884	T/T	A/A	C3	21,234,247	A/A	G/G	C4	10,355,956	T/T	C/C	C5	651,809	C/C	A/A	C5	34,553,537	A/A	G/G	C6	27,103,662	A/A	G/G	C7	37,622,315	C/C	T/T	C8	26,792,669	A/A	C/C	C9	8,414,098	C/C	T/T	C9	53,271,723	T/T	A/A
C1	36,594,164	C/G	T/T	C2	35,106,942	T/T	A/A	C3	21,259,736	G/G	T/T	C4	10,413,996	G/G	A/A	C5	697,074	T/T	G/G	C5	34,737,706	T/T	C/C	C6	27,126,094	T/T	C/C	C7	37,622,318	G/G	A/A	C8	26,825,659	A/A	T/T	C9	8,495,687	T/T	C/C	C9	53,420,110	G/G	T/T
C1	36,614,582	G/G	A/A	C2	35,216,489	T/T	G/G	C3	21,262,107	A/A	C/C	C4	10,414,000	A/A	T/T	C5	697,098	A/A	G/G	C5	34,737,742	G/G	T/T	C6	27,126,126	C/C	A/A	C7	37,622,366	G/G	A/A	C8	26,825,686	A/A	G/G	C9	8,495,693	T/T	C/C	C9	53,487,859	A/A	T/T
C1	36,614,585	A/A	G/G	C2	35,339,210	G/G	A/A	C3	21,311,058	C/C	G/G	C4	10,529,537	A/A	C/C	C5	741,426	C/C	A/A	C5	34,737,760	C/C	A/A	C6	27,522,256	G/G	A/A	C7	37,633,536	A/A	C/C	C8	26,932,085	C/C	A/A	C9	8,495,735	C/C	T/T	C9	53,550,950	T/T	C/C
C1	36,617,564	A/A	T/T	C2	35,426,222	G/G	T/T	C3	21,437,165	A/A	C/G	C4	10,562,630	A/A	G/G	C5	741,437	G/G	A/A	C5	34,751,618	G/A	A/A	C6	27,522,478	T/T	C/C	C7	37,633,552	C/C	T/T	C8	26,932,240	A/A	C/C	C9	8,495,759	A/A	G/G	C9	53,755,548	T/T	C/C
C1	37,223,298	C/C	T/T	C2	35,561,009	T/T	A/A	C3	21,470,163	T/T	A/A	C4	10,562,690	A/A	G/G	C5	803,560	C/C	G/G	C5	34,862,265	A/A	C/C	C6	27,880,901	C/C	T/T	C7	37,633,556	T/T	C/C	C8	26,932,265	G/G	A/A	C9	8,506,584	A/A	T/T	C9	53,755,551	G/G	A/A
C1	37,223,602	G/G	A/A	C2	35,629,296	C/C	G/G	C3	21,532,289	C/C	T/T	C4	10,562,750	A/A	T/T	C5	803,608	A/A	C/C	C5	34,862,319	G/G	A/A	C6	28,020,622	C/C	A/A	C7	37,635,449	A/A	G/G	C8	26,932,266	T/T	A/A	C9	8,506,758	C/C	G/G	C9	53,755,751	T/T	C/C
C1	37,224,316	A/A	T/T	C2	35,629,365	T/T	A/A	C3	21,664,797	C/C	A/A	C4	10,562,925	T/T	C/C	C5	807,548	A/A	T/T	C5	34,866,878	C/C	A/A	C6	28,036,838	A/A	G/G	C7	37,636,151	G/G	A/A	C8	26,981,080	T/T	G/G	C9	8,656,283	G/G	T/T	C9	53,971,661	C/C	G/G
C1	37,224,471	T/T	G/G	C2	35,663,090	T/T	A/A	C3	21,664,810	G/G	C/C	C4	10,562,928	G/G	C/C	C5	807,665	C/C	A/A	C5	34,866,881	G/A	A/A	C6	28,036,888	A/A	G/G	C7	37,743,966	G/G	A/A	C8	26,981,302	C/C	T/T	C9	8,656,351	C/C	T/T	C9			

Appendix II *Cont.***Table S2: *Cont.* : Differences in Allelic variants at various chromosomes positions in parent lines; the cultivated rapid cycling DHSL150 and C07060.**

Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060								
C1	38,040,704	T/T	C/C	C2	36,170,872	A/A	G/G	C3	22,235,860	C/C	T/T	C4	11,403,517	A/A	G/G	C5	1,113,781	A/A	G/G	C6	28,598,160	A/A	G/G	C7	37,875,232	A/A	G/G	C8	27,458,370	G/G	A/A	C9	9,038,429	T/T	G/G	C9	54,272,240	T/T	C/C
C1	38,040,709	T/T	A/A	C2	36,253,835	C/C	G/G	C3	22,259,779	A/A	G/G	C4	11,403,536	G/G	T/T	C5	1,116,319	T/T	A/A	C6	28,598,191	C/C	T/T	C7	37,907,516	C/C	T/T	C8	27,596,569	T/T	C/C	C9	9,072,725	A/A	C/C	C9	54,388,981	A/A	G/G
C1	38,063,722	C/C	T/T	C2	36,331,434	T/T	G/G	C3	22,264,796	A/A	G/G	C4	11,403,595	T/T	G/G	C5	1,116,340	C/C	A/A	C6	28,719,707	G/G	A/A	C7	37,911,389	T/T	G/G	C8	27,606,395	A/A	C/C	C9	9,072,747	T/T	A/A	C9	54,418,685	A/A	G/G
C1	38,063,812	C/C	T/T	C2	36,395,021	C/C	G/G	C3	22,273,601	G/G	A/A	C4	11,654,591	T/T	C/C	C5	1,122,106	A/A	G/G	C6	28,723,457	C/C	T/T	C7	37,912,008	T/T	G/G	C8	27,606,414	G/G	C/C	C9	9,169,036	G/G	A/A	C9	54,435,212	T/T	A/A
C1	38,120,612	C/C	A/A	C2	36,395,035	G/G	A/A	C3	22,338,432	C/C	T/T	C4	11,756,988	T/T	C/C	C5	1,122,217	G/G	T/T	C6	28,736,668	C/C	G/G	C7	37,912,050	G/G	A/A	C8	27,606,658	G/G	A/A	C9	9,249,871	C/C	T/T	C9	54,435,249	T/T	A/A
C1	38,121,210	G/G	T/T	C2	36,464,885	C/C	T/T	C3	22,489,737	A/A	T/T	C4	11,770,139	C/C	T/T	C5	1,138,192	T/T	A/A	C6	28,736,702	G/G	T/T	C7	37,976,975	A/A	G/G	C8	27,606,668	G/G	C/C	C9	9,259,740	A/A	G/G	C9	54,520,882	G/G	A/A
C1	38,121,324	G/G	A/A	C2	36,694,247	G/G	A/A	C3	22,490,042	T/T	C/C	C4	11,835,659	T/T	G/G	C5	1,138,411	A/A	G/A	C6	28,961,704	T/T	G/G	C7	37,976,984	C/C	A/A	C8	27,606,749	C/C	T/T	C9	9,259,749	G/G	C/C	C9	54,537,389	C/C	G/G
C1	38,306,910	C/C	T/T	C2	36,694,273	C/C	A/A	C3	22,490,057	T/T	C/C	C4	11,835,704	T/T	C/C	C5	1,139,902	G/A	G/G	C6	28,961,811	A/A	G/A	C7	37,976,994	T/T	C/C	C8	27,608,330	G/A	A/A	C9	9,286,159	G/G	T/T	C9	54,537,454	A/A	C/C
C1	38,327,863	C/C	A/A	C2	36,694,306	T/T	C/C	C3	22,495,174	T/T	C/C	C4	11,862,060	T/T	C/C	C5	1,248,191	C/C	T/T	C6	28,961,907	G/A	G/C	C7	37,977,095	T/T	C/C	C8	27,651,001	G/G	C/C	C9	9,286,524	T/T	A/A	C9	54,539,951	C/C	T/T
C1	38,327,888	T/T	C/C	C2	36,872,331	A/A	G/G	C3	22,495,182	G/G	A/A	C4	11,862,067	A/A	C/C	C5	1,289,719	C/C	T/T	C6	28,961,934	T/T	G/G	C7	37,977,247	A/A	T/T	C8	27,736,106	C/C	G/G	C9	9,286,536	A/A	T/T	C9	54,546,555	G/G	T/T
C1	38,412,466	C/C	A/A	C2	36,872,401	T/T	C/C	C3	22,495,219	T/T	C/C	C4	11,862,121	C/C	T/T	C5	1,311,152	C/C	T/T	C6	28,974,127	A/A	C/C	C7	37,989,237	G/G	T/T	C8	27,739,594	C/C	T/T	C9	9,287,057	A/A	C/C	C9	54,570,288	A/A	T/T
C1	38,579,761	C/C	A/A	C2	36,891,488	G/G	C/C	C3	22,495,303	A/A	G/G	C4	11,914,577	T/T	C/C	C5	1,354,811	T/T	C/C	C6	28,974,189	C/C	T/T	C7	38,030,209	G/G	A/A	C8	27,747,355	A/A	G/G	C9	9,329,620	A/A	G/G	C9	54,570,300	G/G	A/A
C1	38,713,707	C/C	T/T	C2	36,891,502	G/G	A/A	C3	22,495,327	A/A	C/C	C4	11,940,566	C/C	T/T	C5	1,354,844	A/A	T/T	C6	28,976,069	C/C	A/A	C7	38,030,211	A/A	T/T	C8	27,781,499	A/A	T/T	C9	9,329,633	C/C	T/T	C9	54,628,263	G/G	C/C
C1	38,896,384	A/A	G/G	C2	36,891,510	C/C	T/T	C3	22,495,333	G/G	A/A	C4	12,298,157	T/T	C/C	C5	1,372,715	G/G	T/T	C6	29,232,833	A/C	C/C	C7	38,031,692	G/G	A/A	C8	27,845,358	G/G	A/A	C9	9,329,669	A/A	C/C	C9	54,638,117	T/T	A/A
C1	38,896,495	C/C	T/T	C2	36,968,788	A/A	G/G	C3	22,495,415	C/C	T/T	C4	12,341,997	C/A	A/A	C5	1,372,757	T/T	C/C	C6	29,247,775	A/A	G/G	C7	38,038,358	T/T	A/A	C8	27,880,672	T/T	A/A	C9	9,381,118	T/T	C/C	C9	54,638,123	C/C	G/G
C1	39,033,585	C/C	T/T	C2	36,977,756	T/T	C/C	C3	22,496,140	T/T	C/C	C4	12,421,290	C/C	A/A	C5	1,383,596	C/C	T/T	C6	29,249,335	C/C	A/A	C7	38,038,414	A/A	T/T	C8	27,880,736	A/A	C/C	C9	9,531,819	T/T	C/C				
C1	39,033,621	A/A	G/G	C2	37,143,743	T/T	G/G	C3	22,527,041	T/T	C/C	C4	12,465,618	T/T	G/G	C5	1,386,634	C/A	C/C	C6	29,371,643	G/G	A/A	C7	38,081,421	C/C	T/T	C8	27,880,803	T/T	A/A	C9	9,532,088	T/T	C/C				
C1	39,033,693	T/T	C/C	C2	37,263,716	G/G	T/T	C3	22,527,092	A/A	T/T	C4	12,465,669	A/A	G/G	C5	1,386,646	C/C	T/T	C6	29,371,656	G/G	A/A	C7	38,081,853	G/G	C/C	C8	27,909,705	G/G	A/A	C9	9,532,133	T/T	A/A				
C1	39,033,696	T/T	G/G	C2	37,295,570	T/T	G/G	C3	22,604,101	T/T	G/G	C4	12,465,673	A/A	G/G	C5	1,549,661	G/A	A/A	C6	29,378,988	C/C	T/T	C7	38,086,261	T/T	G/G	C8	27,918,129	G/G	A/A	C9	9,542,509	A/A	G/G				
C1	39,033,789	G/G	T/T	C2	37,295,614	G/G	A/A	C3	22,633,930	A/A	T/T	C4	12,492,042	T/T	C/C	C5	1,549,688	G/G	T/T	C6	29,379,014	T/T	C/C	C7	38,097,808	A/A	C/C	C8	27,918,145	A/A	G/G	C9	9,551,726	G/G	T/T				
C1	39,034,059	T/T	C/C	C2	37,295,618	T/T	A/A	C3	22,633,932	G/G	T/T	C4	12,492,180	G/G	C/C	C5	1,549,694	C/C	T/T	C6	29,393,797	G/G	A/A	C7	38,098,223	A/A	G/G	C8	27,929,479	T/T	G/G	C9	9,587,536	A/A	T/T				
C1	39,034,065	A/A	G/G	C2	37,399,699	A/A	G/G	C3	22,648,279	T/T	C/C	C4	12,492,846	A/A	G/G	C5	1,699,389	A/A	T/T	C6	29,813,889	C/C	T/T	C7	38,136,069	C/C	G/G	C8	28,054,122	A/A	G/G	C9	9,787,542	A/A	G/G				
C1	39,034,125	T/T	C/C	C2	37,498,782	T/T	A/A	C3	22,660,390	T/T	A/A	C4	12,492,945	T/T	C/C	C5	1,699,523	G/G	A/A	C6	29,855,482	C/C	T/T	C7	38,179,906	T/T	C/C	C8	28,054,133	A/A	G/G	C9	9,869,237	A/A	T/T				
C1	39,034,134	C/C	A/A	C2	37,543,283	C/C	T/T	C3	22,680,269	T/T	C/C	C4	12,492,956	A/A	C/C	C5	1,724,433	C/A	A/A	C6	29,903,904	A/A	C/C	C7	38,179,953	T/T	C/C	C8	28,516,908	G/G	A/A	C9	9,869,274	T/T	G/G				
C1	39,248,268	G/G	A/A	C2	37,549,403	T/T	C/C	C3	22,681,403	A/A	G/G	C4	12,496,135	C/C	T/T	C5	1,726,699	G/G	A/A	C6	29,903,953	A/A	G/G	C7	38,197,729	C/C	T/T	C8	28,516,945	A/A	G/G	C9	10,014,761	T/T	C/C				
C1	39,248,343	A/A	G/G	C2	37,590,078	G/G	A/A	C3	22,843,323	A/A	G/G	C4	12,665,458	A/A	G/G	C5	1,735,535	T/T	G/G	C6	29,917,314	T/T	G/G	C7	38,252,101	C/C	T/T	C8	28,620,210	G/G	T/T	C9	10,015,913	A/A	G/G				
C1	39,248,562	G/G	T/T	C2	37,618,477	C/C	T/T	C3	22,963,955	C/C	G/G	C4	12,763,543	A/A	G/G	C5	1,808,471	T/T	C/C	C6	30,342,102	A/A	G/G	C7	38,252,310	T/T	C/C	C8	28,842,603	G/G	T/T	C9	10,020,479	A/A	G/G				
C1	39,378,874	T/T	C/C	C2	37,621,949	G/G	T/T	C3	22,963,963	A/A	T/T	C4	13,148,818	G/G	A/A	C5	1,886,551	T/A	C/C	C6	30,397,185	T/T	C/C	C7	38,277,667	A/A	C/C	C8	28,861,855	A/A	C/C	C9	10,293,690	T/T	G/G				
C1	39,378,891	T/T	C/C	C2	38,128,102	G/G	C/C	C3	22,963,973	G/G	A/A	C4	13,217,154	A/A	G/G	C5	1,886,561	T/T	C/C	C6	30,411,558	A/A	G/G	C7	38,281,074	C/C	G/G	C8	28,880,495	G/G	T/T	C9	10,293,705	C/C	T/T				
C1	39,379,057	A/A	T/T	C2	38,402,146	A/A	G/G	C3	23,075,897	T/T	C/C	C4	13,231,377	C/A	A/A	C5	1,886,566	T/T	C/C	C6	30,411,728	T/T	G/G	C7	38,281,084	A/A	G/G	C8	28,928,765	T/T	C/C	C9	10,296,996	A/A	G/G				
C1	39,441,121	T/T	C/C	C2	38,577,660	G/G	C/C	C3	23,122,939	C/C	T/T	C4	13,232,142	C/C	T/T	C5	1,907,846	C/C	T/T	C6	30,486,553	T/T	C/C	C7	38,434,411	T/T	C/C	C8	29,036,167	C/C	G/G	C9	10,297,127	A/A	G/G				
C1	39,441,158	A/A	G/G	C2	38,577,709	G/G	T/T	C3	23,356,680	C/C	T/T	C4	13,233,119	A/A	G/G	C5	1,925,663	T/T	A/A	C6	30,512,673	G/G	C/C	C7	38,434,442	T/T	C/C	C8	29,063,059	T/T	C/C	C9	10,409,146	A/A	G/G				
C1	39,490,316	G/G	A/A	C2	38,590,100	C/C	T/T	C3	23,412,314	G/G	C/C	C4	13,353,104	G/G	C/C	C5	1,925,666	C/C	T/T	C6	30,662,123	A/A	C/C	C7	38,434,528	G/G	C/C	C8	29,063,105	G/G	A/A	C9	10,409,208	T/T	G/G				
C1	39,505,296	G/G	A/A	C2	38,590,126	A/A	T/T	C3	23,412,322	C/C	T/T	C4	13,512,949	C/C	A/A	C5	1,925,687	G/G	A/A	C6	30,695,420	A/A	G/G	C7	38,434,618	T/T	C/C	C8	29,063,162	T/T	A/A	C9	10,409,243	G/G	A/A				
C1	39,592,946	A/A	G/G	C2	38,621,907	A/A	C/C	C3	23,412,346	C/C	G/G	C4	13,630,205	T/T	A/A	C5	1,925,758	C/A	T/T	C6	30,695,429	A/A	G/G	C7	38,438,186	C/C	T/T	C8	29,419,058	A/A	G/G	C9	10,409,300	G/G	A/A				
C1	39,615,777	A/A	T/T	C2	38,712,958	A/A	G/G	C3	23,465,764	C/A	A/A	C4	13,630,238	A/A	C/C	C5	1,925,762	C/C	T/T	C6	30,695,498	T/T	C/C	C7	38,438,187	A/A	C/C	C8	29,485,448	A/A	T/T	C9	10,409,312	T/T	A/A				
C1	39,681,452	C/C	A/A	C2	38,894,235	C/C	T/T	C3	23,496,017	G/G	A/A	C4	13,769,593	C/A	A/A	C5	1,926,943	T/T	C/C	C6	30,809,046	C/A	A/A	C7	38,455,399	A/A	C/C	C8	29,485,466	T/T	C/C	C9	10,568,945	T/T	G/G				
C1	39,681,607	A/A	T/T	C2	38,894,248	T/T	C/C	C3	23,496,023	C/A	A/A	C4	13,769,719	C/C	A/A	C5	1,969,381	G/A	A/A	C6	30,994,740	T/T	A/A	C7	38,455,420	T/T	A/A	C8	29,753,179	G/G	T/T	C9	10,569,640	T/T	G/G				
C1	39,681,824	A/A	G/G	C																																			

Appendix II Cont.

Table S2: Cont. : Differences in Allelic variants at various chromosomes positions in parent lines; the cultivated rapid cycling DHSL150 and C07060.

Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060								
C1	40,259,216	G/G	C/C	C2	40,862,050	C/C	T/T	C3	24,196,547	A/A	C/C	C4	14,504,173	C/C	T/T	C5	2,652,767	T/T	C/C	C5	38,756,341	T/T	G/G	C6	32,150,560	T/T	A/A	C7	38,691,315	T/T	C/C	C8	30,604,043	T/T	G/G	C9	11,336,649	C/C	G/G
C1	40,259,988	T/T	C/C	C2	41,301,306	T/T	C/C	C3	24,196,565	C/C	G/G	C4	14,504,182	T/T	A/A	C5	2,663,244	C/C	A/A	C5	38,756,386	A/A	C/C	C6	32,150,588	C/C	T/T	C7	38,697,485	G/G	T/T	C8	30,643,171	A/A	C/C	C9	11,485,399	A/A	C/C
C1	40,278,926	T/T	G/G	C2	41,301,310	T/T	A/A	C3	24,342,328	T/T	C/C	C4	14,541,936	C/C	T/T	C5	2,663,282	C/C	T/T	C5	38,758,455	G/G	A/A	C6	32,164,273	G/G	C/C	C7	38,697,503	A/A	G/G	C8	30,643,174	T/T	A/A	C9	11,499,383	A/A	C/C
C1	40,322,882	T/T	A/A	C2	41,580,892	C/C	G/G	C3	24,389,965	C/C	G/G	C4	14,604,076	T/T	C/C	C5	2,713,712	T/T	G/G	C5	38,790,210	A/A	G/G	C6	32,254,163	G/G	C/C	C7	38,773,041	C/C	G/G	C8	30,643,198	T/T	A/A	C9	11,499,421	A/A	C/C
C1	40,474,735	G/G	T/T	C2	41,580,896	G/G	T/T	C3	24,390,022	C/C	T/T	C4	14,604,095	G/G	A/A	C5	2,816,010	A/A	C/C	C5	38,794,775	A/A	G/G	C6	32,316,939	C/C	T/T	C7	38,826,119	C/C	T/T	C8	30,646,491	G/G	T/T	C9	11,499,425	T/T	G/G
C1	40,495,110	C/C	T/T	C2	41,625,120	G/G	C/C	C3	24,447,923	C/C	T/T	C4	14,611,167	A/A	T/T	C5	2,816,011	A/A	C/C	C5	38,794,940	C/C	A/A	C6	32,426,711	C/C	T/T	C7	38,826,140	G/G	C/C	C8	30,646,514	T/T	C/C	C9	11,514,474	C/C	T/T
C1	40,542,660	C/C	G/G	C2	41,625,127	T/T	C/C	C3	24,526,675	G/G	T/T	C4	14,611,175	T/T	G/G	C5	2,816,046	T/T	C/C	C5	38,915,602	G/G	A/A	C6	32,427,222	T/T	C/T	C7	38,854,502	G/G	T/T	C8	30,685,417	G/G	A/A	C9	11,514,568	A/A	G/G
C1	40,722,751	T/T	C/C	C2	41,625,133	T/T	A/A	C3	24,526,732	C/C	G/G	C4	14,887,403	G/G	A/A	C5	2,816,095	C/C	A/A	C5	38,951,768	C/C	A/A	C6	32,581,136	A/A	G/G	C7	38,855,486	G/G	A/A	C8	30,690,376	T/T	C/C	C9	11,570,291	A/A	G/G
C1	40,765,905	T/T	C/C	C2	41,625,198	T/T	G/G	C3	24,776,748	G/G	C/C	C4	14,904,480	G/G	T/T	C5	2,816,126	T/T	A/A	C5	38,951,805	T/T	A/A	C6	32,602,756	C/C	A/A	C7	38,855,498	G/G	A/A	C8	30,690,381	T/T	A/A	C9	11,570,309	G/G	A/A
C1	40,768,546	A/A	G/G	C2	41,700,619	A/A	C/C	C3	24,802,802	T/T	C/C	C4	14,915,794	C/C	A/A	C5	2,893,159	G/G	A/A	C5	38,951,912	T/T	G/G	C6	32,635,499	A/A	G/G	C7	38,862,327	T/T	A/A	C8	30,728,250	T/T	G/G	C9	11,570,444	C/C	A/A
C1	40,824,790	C/C	T/T	C2	41,701,135	G/G	T/T	C3	24,802,837	C/C	T/T	C4	15,364,148	T/T	G/G	C5	2,893,174	A/A	G/G	C5	38,952,153	C/C	A/A	C6	32,776,603	C/C	T/T	C7	38,862,414	G/G	A/A	C8	30,937,104	A/A	G/G	C9	11,570,637	C/C	T/T
C1	40,916,156	C/C	T/T	C2	41,701,141	T/T	A/A	C3	24,841,255	C/C	T/T	C4	15,590,917	G/G	C/C	C5	2,893,178	A/A	C/C	C5	38,952,584	G/G	A/A	C6	32,777,992	C/C	G/G	C7	39,094,162	C/C	T/T	C8	31,069,978	T/T	C/C	C9	11,661,933	C/C	T/T
C1	40,971,471	C/C	T/T	C2	41,701,149	T/T	C/C	C3	24,843,214	T/T	G/G	C4	15,603,356	T/T	C/C	C5	2,893,312	C/C	T/T	C5	38,952,657	A/A	T/T	C6	32,778,675	G/G	A/A	C7	39,094,226	C/C	A/A	C8	31,078,021	T/T	A/A	C9	11,661,989	G/G	T/T
C1	40,971,482	T/T	A/A	C2	41,701,204	G/G	A/A	C3	24,898,414	C/C	T/T	C4	15,603,396	C/C	G/G	C5	2,925,177	G/G	A/A	C5	38,952,889	A/A	G/G	C6	32,810,626	T/T	G/G	C7	39,239,117	C/C	T/T	C8	31,084,096	G/G	A/A	C9	12,434,276	T/T	C/C
C1	40,971,571	T/T	C/C	C2	41,832,873	G/G	A/A	C3	24,898,461	C/C	T/T	C4	15,630,322	T/T	G/G	C5	2,930,649	G/G	T/T	C5	38,953,107	C/C	T/T	C6	32,810,752	T/T	C/C	C7	39,305,624	A/A	G/G	C8	31,206,415	G/G	T/T	C9	12,450,351	G/G	A/A
C1	41,006,800	T/T	C/C	C2	41,834,087	T/T	G/G	C3	24,899,586	C/C	T/T	C4	15,709,489	T/T	G/G	C5	2,930,703	T/T	C/C	C5	38,953,773	A/A	T/T	C6	32,810,910	A/A	C/C	C7	39,340,455	G/G	A/A	C8	31,351,701	C/C	A/A	C9	12,450,487	G/G	A/A
C1	41,012,675	G/G	A/A	C2	41,835,838	G/G	T/T	C3	24,912,207	A/A	T/T	C4	15,709,507	C/C	G/G	C5	2,956,549	C/C	T/T	C5	38,987,082	G/G	A/A	C6	33,017,770	T/T	C/C	C7	39,340,566	C/C	T/T	C8	31,471,869	C/C	T/T	C9	12,458,770	G/G	A/A
C1	41,021,383	A/A	G/G	C2	42,240,693	G/G	C/C	C3	24,912,287	T/T	A/A	C4	15,729,560	T/T	C/C	C5	2,956,569	T/T	A/A	C5	38,987,146	G/G	A/A	C6	33,017,782	C/C	T/T	C7	39,396,581	A/A	T/T	C8	31,557,221	G/G	A/A	C9	12,660,143	A/A	G/G
C1	41,129,237	T/T	G/G	C2	42,340,979	T/T	G/G	C3	24,925,264	A/A	G/G	C4	15,729,589	G/G	A/A	C5	2,959,940	G/G	C/C	C5	38,989,531	G/G	C/C	C6	33,019,640	A/A	G/G	C7	39,396,590	C/C	T/T	C8	31,613,406	T/T	C/C	C9	13,327,205	C/C	G/G
C1	41,129,552	C/C	T/T	C2	42,340,983	T/T	C/C	C3	24,950,764	T/T	C/C	C4	15,729,628	A/A	G/G	C5	3,020,649	C/C	T/T	C5	38,989,625	T/T	C/C	C6	33,037,563	C/C	A/A	C7	39,396,676	A/A	C/C	C8	31,613,427	A/A	G/G	C9	13,408,403	G/G	T/T
C1	41,348,955	G/G	A/A	C2	42,345,754	C/C	A/A	C3	24,950,791	T/T	C/C	C4	15,730,046	T/T	C/C	C5	3,121,043	C/C	T/T	C5	38,989,663	T/T	G/G	C6	33,121,043	G/G	A/A	C7	39,397,099	T/T	A/A	C8	31,613,442	G/G	T/T	C9	13,642,380	C/C	A/A
C1	41,349,195	A/A	G/G	C2	42,345,762	T/T	A/A	C3	24,961,808	C/C	A/A	C4	15,730,069	T/T	A/A	C5	3,123,732	C/C	G/G	C5	39,017,301	T/T	C/C	C6	33,086,885	C/C	A/A	C7	39,419,556	C/C	T/T	C8	31,616,251	A/A	G/G	C9	13,679,900	C/C	T/T
C1	41,392,495	A/A	G/G	C2	42,608,821	C/C	A/A	C3	24,961,820	A/A	C/C	C4	15,808,937	G/G	A/A	C5	3,157,070	G/G	A/A	C5	39,104,134	G/G	C/C	C6	33,121,576	A/A	G/G	C7	39,419,577	G/G	T/T	C8	31,616,264	G/G	A/A	C9	13,699,499	G/G	G/G
C1	41,414,562	T/T	C/C	C2	42,608,847	G/G	A/A	C3	24,961,871	G/G	A/A	C4	15,812,372	G/G	A/A	C5	3,186,622	C/C	A/A	C5	39,104,145	T/T	C/C	C6	33,194,364	G/G	A/A	C7	39,480,957	T/T	A/A	C8	31,655,387	C/C	A/A	C9	13,752,617	G/G	A/A
C1	41,414,604	C/C	T/T	C2	42,647,466	A/A	T/T	C3	24,963,199	T/T	A/A	C4	16,103,359	C/C	T/T	C5	3,186,637	A/A	T/T	C5	39,104,733	A/A	G/G	C6	33,456,761	T/T	G/G	C7	39,530,721	A/A	T/T	C8	31,655,426	A/A	G/G	C9	13,802,026	A/A	G/G
C1	41,414,613	G/G	A/A	C2	42,750,659	C/C	A/A	C3	24,963,277	G/G	A/A	C4	16,142,813	T/T	G/G	C5	3,275,253	C/C	A/A	C5	39,122,417	T/T	C/C	C6	33,499,558	G/G	A/A	C7	39,759,056	C/C	T/T	C8	31,699,357	A/A	G/G	C9	13,826,968	C/C	T/T
C1	41,498,294	C/C	T/T	C2	43,177,062	G/G	T/T	C3	24,972,779	A/A	G/G	C4	16,159,114	A/A	G/G	C5	3,275,270	G/G	A/A	C5	39,125,417	T/T	C/C	C6	33,525,385	A/A	G/G	C7	39,782,802	C/C	T/T	C8	31,699,365	C/C	T/T	C9	13,826,985	A/A	G/G
C1	41,498,334	A/A	G/G	C2	43,220,504	T/T	A/A	C3	24,972,833	A/A	T/T	C4	16,268,543	C/C	G/G	C5	3,318,677	A/A	C/C	C5	39,125,442	C/C	T/T	C6	33,678,557	G/G	A/A	C7	39,797,842	A/A	G/G	C8	31,704,195	C/C	T/T	C9	13,827,003	A/A	C/C
C1	41,498,455	C/C	T/T	C2	43,231,640	T/T	C/C	C3	24,974,435	A/A	G/G	C4	16,372,880	T/T	C/C	C5	3,336,797	T/T	C/C	C5	39,125,844	T/T	C/C	C6	33,826,415	A/A	C/C	C7	39,797,854	A/A	T/T	C8	31,704,218	T/T	C/C	C9	13,935,936	A/A	G/G
C1	41,498,479	A/A	C/C	C2	43,328,636	T/T	G/G	C3	24,984,415	T/T	A/A	C4	16,527,270	G/G	C/C	C5	3,341,833	T/T	C/C	C5	39,125,883	T/T	C/C	C6	34,021,107	C/C	T/T	C7	39,878,744	T/T	C/C	C8	31,704,304	T/T	G/G	C9	13,951,010	T/T	G/G
C1	41,498,522	G/G	T/T	C2	43,381,025	C/C	A/A	C3	24,985,887	G/G	A/A	C4	16,680,692	C/C	G/G	C5	3,358,601	A/A	T/T	C5	39,125,907	T/T	C/C	C6	34,021,127	A/A	G/G	C7	39,912,180	T/T	G/G	C8	31,704,318	A/A	G/G	C9	13,951,012	T/T	C/C
C1	41,504,108	T/T	C/C	C2	43,381,031	C/C	T/T	C3	24,986,045	A/A	T/T	C4	16,689,965	C/C	T/T	C5	3,358,616	A/A	G/G	C5	39,134,529	G/G	A/A	C6	34,046,259	C/C	T/T	C7	39,912,188	G/G	A/A	C8	31,848,773	A/A	C/C	C9	13,975,859	A/A	G/G
C1	41,563,021	G/G	A/A	C2	43,381,065	C/A	A/A	C3	24,986,084	G/G	A/A	C4	16,690,031	C/A	G/G	C5	3,458,927	A/A	C/C	C5	39,135,148	G/G	C/C	C6	34,046,324	A/A	G/G	C7	40,042,042	A/A	G/G	C8	31,874,202	T/T	C/C	C9	13,979,893	A/A	T/T
C1	41,563,042	G/G	A/A	C2	43,381,083	A/A	G/G	C3	25,007,821	T/T	A/A	C4	16,749,384	G/G	A/A	C5	3,554,602	G/G	A/A	C5	39,135,167	G/G	A/A	C6	34,046,336	A/A	G/G	C7	40,352,226	A/A	G/G	C8	31,874,230	T/T	G/G	C9	14,025,530	G/G	A/A
C1	41,563,085	T/T	C/C	C2	43,382,407	A/A	G/G	C3	25,007,830	T/T	A/A	C4	16,749,544	A/A	G/G	C5	3,554,605	G/G	A/A	C5	39,139,373	A/A	C/C	C6	34,057,117	A/A	G/G	C7	40,393,232	C/C	T/T	C8	31,985,924	C/C	T/T	C9	14,131,650	C/C	A/A
C1	41,563,126	G/G	C/C	C2	43,382,420	A/A	G/G	C3	25,007,880	G/G	A/A	C4	16,781,826	A/A	G/G	C5	3,574,901	C/C	G/G	C5	39,139,393	C/C	A/A	C6	34,057,132	A/A	G/G	C7	40,458,663	A/A	T/T								

Appendix II Cont.

Table S2: Cont. : Differences in Allelic variants at various chromosomes positions in parent lines; the cultivated rapid cycling DHSL150 and C07060.

Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060	Chr.	Position	DHSL150	C07060				
C1	41,932,605	T/T	C/C	C2	43,537,340	T/T	A/A	C3	25,307,890	G/G	A/A	C4	18,002,431	T/T	C/C	C5	3,754,245	G/G	A/A	C6	34,097,416	G/G	T/T	C7	41,180,016	C/C	T/T	C8	32,435,737	G/G	A/A	C9	14,684,938	A/A	T/T
C1	42,103,028	A/A	G/G	C2	43,547,349	A/A	G/G	C3	25,354,841	A/A	G/G	C4	18,002,523	C/C	G/G	C5	3,754,285	G/G	T/T	C6	34,308,153	T/T	G/G	C7	41,181,152	T/T	A/A	C8	32,444,343	T/T	C/C	C9	14,817,926	A/A	G/G
C1	42,103,069	C/C	T/T	C2	43,654,302	A/A	C/C	C3	25,355,406	T/T	G/G	C4	18,092,687	A/A	G/G	C5	3,754,362	C/C	A/A	C6	34,308,204	C/C	T/T	C7	41,181,208	G/A	A/A	C8	32,444,375	C/C	A/A	C9	15,095,254	C/C	T/T
C1	42,103,137	T/T	C/C	C2	43,769,436	G/G	A/A	C3	25,421,524	G/G	C/C	C4	18,440,987	T/T	A/A	C5	3,754,377	G/G	A/A	C6	34,315,438	T/T	C/C	C7	41,181,212	C/C	G/G	C8	32,593,295	A/A	G/G	C9	15,332,589	T/T	G/G
C1	42,248,858	G/G	A/A	C2	43,794,034	A/A	G/G	C3	25,458,009	A/A	G/G	C4	18,741,108	T/T	A/A	C5	3,815,747	G/G	C/C	C6	34,329,343	T/T	C/C	C7	41,181,238	G/G	A/A	C8	32,642,148	T/T	C/C	C9	15,363,747	G/G	A/A
C1	42,257,377	G/G	A/A	C2	43,932,381	A/A	G/G	C3	25,498,048	C/C	G/G	C4	18,791,618	A/A	G/G	C5	3,857,858	A/A	G/G	C6	34,502,347	A/A	C/C	C7	41,200,507	C/C	A/A	C8	32,772,430	G/G	C/C	C9	15,642,294	C/C	A/A
C1	42,361,150	G/G	A/A	C2	44,044,281	T/T	C/C	C3	25,685,686	T/T	A/A	C4	19,281,036	C/C	T/T	C5	3,857,864	A/A	G/G	C6	34,521,883	T/T	C/C	C7	41,203,213	T/T	G/G	C8	32,772,457	A/A	G/G	C9	15,646,244	G/G	A/A
C1	42,362,847	G/G	A/A	C2	44,044,283	T/T	C/C	C3	26,101,543	T/T	C/C	C4	19,318,950	T/T	C/C	C5	3,857,873	C/C	T/T	C6	34,711,347	T/T	C/C	C7	41,290,901	A/A	T/T	C8	32,772,905	T/T	C/C	C9	15,646,253	A/A	G/G
C1	42,362,853	G/G	A/A	C2	44,044,408	T/T	A/A	C3	26,101,546	C/C	T/T	C4	19,385,313	A/A	G/G	C5	3,941,565	A/A	G/G	C6	34,713,559	C/C	T/T	C7	41,290,913	T/T	A/A	C8	32,775,830	C/C	T/T	C9	15,673,755	G/G	A/A
C1	42,416,410	C/C	G/G	C2	44,044,836	C/C	A/A	C3	26,150,434	A/A	T/T	C4	19,386,930	A/A	C/C	C5	3,977,194	T/T	C/C	C6	34,713,601	A/A	G/G	C7	41,297,469	C/C	T/T	C8	32,818,900	C/C	T/T	C9	15,687,033	A/A	C/C
C1	42,429,060	G/G	A/A	C2	44,044,864	T/T	C/C	C3	26,294,815	G/G	A/A	C4	19,569,437	T/T	C/C	C5	3,977,228	C/A	A/A	C6	34,713,919	G/G	A/A	C7	41,297,845	T/T	G/G	C8	32,897,224	G/G	T/T	C9	15,687,064	C/C	G/G
C1	42,429,203	T/T	C/C	C2	44,276,255	C/C	T/T	C3	26,436,488	T/T	G/G	C4	19,699,506	T/T	G/G	C5	4,021,431	C/A	A/A	C6	34,713,958	A/A	G/G	C7	41,352,445	A/A	C/C	C8	32,912,786	C/C	G/G	C9	15,704,226	G/G	A/A
C1	42,578,236	A/A	G/G	C2	44,318,368	G/G	C/C	C3	26,437,539	T/T	C/C	C4	19,699,743	A/A	G/G	C5	4,027,104	G/A	T/T	C6	34,714,204	C/C	T/T	C7	41,352,452	T/T	C/C	C8	33,348,366	A/A	G/G	C9	15,706,691	C/C	G/G
C1	42,602,512	C/C	G/G	C2	44,318,377	T/T	C/C	C3	26,438,277	T/T	A/A	C4	19,699,759	C/A	A/A	C5	4,027,136	T/T	C/C	C6	34,714,228	T/T	C/C	C7	41,352,488	T/T	A/A	C8	33,383,297	A/A	G/G	C9	16,499,357	T/T	C/C
C1	42,604,779	G/G	C/C	C2	44,720,126	G/G	C/C	C3	26,438,315	C/C	T/T	C4	19,888,677	A/A	G/G	C5	4,027,139	G/A	G/G	C6	34,714,237	G/G	A/A	C7	41,356,951	C/C	G/G	C8	33,385,355	T/T	A/A	C9	16,526,070	G/G	A/A
C1	42,604,784	C/C	G/G	C2	44,720,138	T/T	G/G	C3	26,438,438	T/T	C/C	C4	20,064,456	A/A	C/C	C5	4,027,190	A/A	G/G	C6	34,722,690	T/T	C/C	C7	41,488,911	C/C	A/A	C8	33,432,076	G/G	A/A	C9	16,544,971	T/T	C/C
C1	42,604,901	A/A	G/G	C2	44,781,325	C/C	T/T	C3	26,438,579	T/T	A/A	C4	20,399,754	C/C	T/T	C5	4,027,205	T/T	A/A	C6	34,872,943	A/A	G/G	C7	41,489,078	A/A	G/G	C8	33,434,246	C/A	A/A	C9	16,544,974	T/T	C/C
C1	42,604,921	C/C	T/T	C2	44,884,648	T/T	C/C	C3	26,896,422	C/C	T/T	C4	20,401,455	A/A	G/G	C5	4,027,292	G/A	A/A	C6	34,872,882	G/G	A/A	C7	41,522,002	G/G	A/A	C8	33,550,993	A/A	C/C	C9	16,552,494	A/A	G/G
C1	42,605,505	A/A	T/T	C2	45,007,831	C/C	T/T	C3	27,032,945	A/A	G/G	C4	20,401,554	A/A	G/G	C5	4,027,925	C/A	A/A	C6	34,898,495	G/G	C/C	C7	41,551,570	A/A	G/G	C8	33,719,780	G/G	C/C	C9	16,628,479	C/C	G/G
C1	42,605,724	G/G	A/A	C2	45,007,844	T/T	C/C	C3	27,032,963	G/G	A/A	C4	20,472,645	T/T	C/C	C5	4,027,961	C/A	T/A	C6	34,898,504	A/A	C/C	C7	41,551,594	A/A	G/G	C8	33,720,492	G/G	C/C	C9	16,637,494	C/C	T/T
C1	42,627,652	A/A	C/C	C2	45,007,862	T/T	C/C	C3	27,064,347	A/A	C/C	C4	20,638,804	T/T	C/C	C5	4,028,575	C/A	T/T	C6	34,898,510	C/A	A/A	C7	41,551,671	G/G	A/A	C8	33,720,523	A/A	C/C	C9	16,637,522	C/C	G/G
C1	42,647,083	C/C	G/G	C2	45,007,911	A/A	T/T	C3	27,115,382	A/A	G/G	C4	20,770,427	C/C	G/G	C5	4,028,623	A/A	C/C	C6	34,898,540	A/A	G/G	C7	41,551,784	A/A	G/G	C8	33,801,082	C/C	T/T	C9	16,732,052	C/C	T/T
C1	42,701,903	G/G	C/C	C2	45,406,154	A/A	G/G	C3	27,245,571	G/G	A/A	C4	20,776,603	T/T	A/A	C5	4,029,541	T/T	C/C	C6	35,040,823	T/T	G/G	C7	41,636,985	T/T	C/C	C8	33,801,090	A/A	G/G	C9	16,762,920	G/G	C/C
C1	42,702,473	A/A	G/G	C2	45,415,054	G/G	A/A	C3	27,245,587	T/T	A/A	C4	20,776,604	T/T	G/G	C5	4,029,559	C/A	T/T	C6	35,040,855	C/C	T/T	C7	41,637,182	G/G	A/A	C8	33,803,708	C/C	T/T	C9	16,808,072	G/G	A/A
C1	42,702,574	T/T	G/G	C2	45,453,860	G/G	C/C	C3	27,245,588	A/A	C/C	C4	20,841,300	G/G	A/A	C5	4,066,845	T/T	C/C	C6	35,117,634	A/A	G/G	C7	41,637,795	G/G	C/C	C8	33,803,739	A/A	C/C	C9	16,838,973	C/C	T/T
C1	42,702,615	T/T	A/A	C2	45,501,169	T/T	C/C	C3	27,371,369	A/A	C/C	C4	21,438,679	T/T	G/G	C5	4,170,881	C/C	T/T	C6	35,138,025	G/G	A/A	C7	41,681,508	A/A	T/T	C8	33,804,700	C/C	G/G	C9	16,867,781	G/G	T/T
C1	42,730,714	C/T	A/A	C2	45,502,636	A/A	G/G	C3	27,373,475	G/G	C/C	C4	21,521,374	A/A	G/G	C5	4,238,322	C/C	G/G	C6	35,235,300	T/T	C/C	C7	41,847,676	T/T	C/C	C8	33,804,788	C/C	T/T	C9	16,869,041	G/G	A/A
C1	42,763,380	C/C	T/T	C2	45,689,894	G/G	A/A	C3	27,373,494	T/T	C/C	C4	21,769,771	C/A	A/A	C5	4,248,705	C/A	T/T	C6	35,386,444	A/A	C/C	C7	41,854,104	G/G	A/A	C8	33,804,823	A/A	T/T	C9	16,879,041	C/C	T/T
C1	42,763,428	C/C	T/T	C2	45,830,121	G/G	A/A	C3	27,375,701	T/T	C/C	C4	21,909,763	G/G	C/C	C5	4,248,749	C/C	T/T	C6	35,386,465	G/G	C/C	C7	41,854,136	A/A	G/G	C8	33,804,888	C/C	T/T	C9	16,905,245	A/A	G/G
C1	42,800,307	A/A	G/G	C2	45,830,241	T/T	A/A	C3	27,381,895	T/T	C/C	C4	21,971,713	A/A	T/T	C5	4,387,981	C/C	T/T	C6	35,454,592	T/T	A/A	C7	41,854,186	C/C	A/A	C8	33,807,181	G/G	A/A	C9	17,014,113	A/A	G/G
C1	42,800,384	A/A	T/T	C2	45,967,534	A/A	T/T	C3	27,428,236	G/G	C/C	C4	21,971,789	A/A	T/T	C5	4,429,293	C/C	T/T	C6	35,454,645	G/G	T/T	C7	41,888,148	A/A	T/T	C8	33,807,280	G/G	C/C	C9	17,166,109	T/T	G/G
C1	42,869,982	G/G	A/A	C2	45,967,560	C/C	T/T	C3	27,428,268	T/T	C/C	C4	22,035,522	G/G	A/A	C5	4,468,147	G/G	T/T	C6	35,588,750	C/C	T/T	C7	41,975,577	C/C	T/T	C8	33,831,660	A/A	T/T	C9	17,246,666	A/A	G/G
C1	42,869,983	G/G	C/C	C2	46,200,191	C/C	G/G	C3	27,428,306	A/A	T/T	C4	22,084,782	C/C	A/A	C5	4,468,174	A/A	G/G	C6	35,600,312	A/A	G/G	C7	41,983,988	T/T	C/C	C8	33,833,099	T/T	C/C	C9	17,305,750	C/C	T/T
C1	42,912,404	A/A	G/G	C2	46,366,234	A/A	G/G	C3	27,841,199	C/C	T/T	C4	22,211,422	T/T	C/C	C5	4,494,323	G/G	A/A	C6	35,697,567	G/G	A/A	C7	41,992,082	A/A	G/G	C8	33,833,185	A/A	G/G	C9	17,306,377	A/A	G/G
C1	42,944,591	A/A	G/G	C2	46,366,302	G/G	A/A	C3	27,841,202	G/C	C/C	C4	22,356,776	G/C	C/C	C5	4,494,327	G/A	A/A	C6	35,702,458	C/C	G/G	C7	42,043,473	T/T	G/G	C8	33,833,261	A/A	G/G	C9	17,306,392	T/T	C/C
C1	43,038,968	T/T	G/G	C2	46,366,304	A/A	G/G	C3	27,855,855	A/A	G/G	C4	22,356,777	T/T	A/A	C5	4,494,365	C/A	T/T	C6	35,702,532	C/C	T/T	C7	42,054,883	A/A	G/G	C8	33,833,361	A/A	G/G	C9	17,306,422	G/G	A/A
C1	43,051,951	G/G	A/A	C2	46,429,696	C/C	T/T	C3	27,866,482	G/G	T/T	C4	22,949,090	G/C	C/C	C5	4,503,217	T/T	A/A	C6	35,736,681	C/C	T/T	C7	42,057,564	G/G	A/A	C8	33,837,275	G/G	A/A	C9	17,368,172	T/T	G/G
C1	43,052,092	A/A	G/G	C2	46,827,854	T/T	C/C	C3	27,866,483	A/A	T/T	C4	22,949,156	T/T	C/C	C5	4,503,253	G/G	T/T	C6	35,736,702	A/A	C/C	C7	42,072,650	C/C	T/T	C8	33,837,338	G/G	C/C	C9	17,368,181	A/A	G/G
C1	43,057,045	C/C	G/G	C2	46,998,180	G/G	A/A	C3	27,887,304	G/G	C/C	C4	23,028,948	C/C	G/G	C5	4,503,256	C/C	G/G	C6	35,841,767	C/C	T/T	C7	42,139,168	G/G	A/A	C8	33,863,621	G/G	T/T	C9	17,392,905	C/C	T/T
C1	43,095,016	T/T	A/A	C2	47,135,874	C/C	A/A	C3	27,887,330	A/A	G/G	C4	23,067,898	A/A	T/T	C5	4,503,303	A/A	C/C	C6	35,898,638	C/C	T/T	C7	42,139,264										

Appendix III

Table S3: Differences in Allelic variants at various chromosomes positions in parent lines; the cultivated rapid cycling DHSL150 and C07007.

Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007												
C1	238,942	A/A	C/C	C2	65,478	A/A	C/C	C2	45,415,054	G/G	A/A	C3	26,294,834	C/C	A/A	C4	16,878,327	C/C	A/A	C5	8,025,017	T/T	G/G	C6	7,890,061	T/T	A/A	C7	20,783,233	C/C	T/T	C8	10,855,892	T/T	C/C	C9	2,481,182	T/T	G/G	C9	50,979,561	G/G	T/T
C1	238,943	G/G	T/T	C2	65,497	A/A	T/T	C2	45,453,880	G/G	C/C	C3	26,294,835	C/C	T/T	C4	16,880,692	C/C	G/G	C5	8,025,144	A/A	G/G	C6	7,700,384	G/G	C/C	C7	21,271,174	T/T	C/C	C8	10,855,794	C/C	T/T	C9	2,486,384	A/A	C/C	C9	51,130,063	G/G	T/T
C1	239,032	G/G	A/A	C2	65,519	A/A	G/G	C2	45,501,169	T/T	C/C	C3	26,309,591	C/C	T/T	C4	16,889,965	C/C	T/T	C5	8,031,302	G/G	C/C	C6	7,700,405	G/G	A/A	C7	21,271,242	G/G	T/T	C8	10,893,539	A/A	T/T	C9	2,491,078	T/T	C/C	C9	51,202,802	G/G	C/C
C1	285,728	T/T	C/C	C2	88,295	C/C	T/T	C2	45,502,636	A/A	G/G	C3	26,349,133	G/G	A/A	C4	16,890,031	C/C	G/G	C5	8,042,988	T/T	A/A	C6	7,844,050	G/G	A/A	C7	21,271,248	G/G	C/C	C8	11,035,058	T/T	G/G	C9	2,516,311	C/C	T/T	C9	51,223,456	A/A	C/C
C1	285,786	G/G	A/A	C2	92,240	A/A	G/G	C2	45,535,525	A/A	G/G	C3	26,438,277	T/T	A/A	C4	16,749,384	G/G	A/A	C5	8,043,021	G/G	A/A	C6	7,847,422	T/T	A/A	C7	21,292,479	T/T	C/C	C8	11,102,535	T/T	G/G	C9	2,570,969	A/A	G/G	C9	51,293,463	G/G	A/A
C1	467,022	A/A	G/G	C2	92,267	A/A	C/C	C2	45,536,356	A/A	G/G	C3	26,438,315	C/C	T/T	C4	16,749,544	A/A	G/G	C5	8,046,509	G/G	A/A	C6	7,847,475	G/G	A/A	C7	21,292,488	A/A	C/C	C8	11,276,367	C/C	T/T	C9	2,682,728	A/A	T/T	C9	51,323,557	T/T	C/C
C1	467,057	G/G	A/A	C2	92,399	G/G	A/A	C2	45,542,439	G/G	T/T	C3	26,438,579	T/T	A/A	C4	20,472,645	T/T	C/C	C5	8,046,510	C/C	A/A	C6	7,868,508	G/G	A/A	C7	21,554,102	C/C	T/T	C8	11,276,395	T/T	G/G	C9	2,682,747	G/G	A/A	C9	51,342,477	T/T	A/A
C1	561,602	C/C	A/A	C2	92,424	A/A	C/C	C2	45,542,509	C/C	G/G	C3	26,596,044	A/A	G/G	C4	20,472,683	C/C	T/T	C5	8,052,971	T/T	C/C	C6	7,884,030	A/A	G/G	C7	21,554,110	T/T	G/G	C8	11,277,610	C/C	T/T	C9	2,685,872	A/A	G/G	C9	51,342,563	T/T	C/C
C1	647,111	C/C	T/T	C2	209,449	C/C	A/A	C2	45,937,159	T/T	A/A	C3	27,032,945	A/A	G/G	C4	22,713,435	T/T	C/C	C5	8,055,172	G/G	A/A	C6	7,912,507	G/G	C/C	C7	21,554,151	G/G	C/C	C8	11,340,738	G/G	A/A	C9	2,712,281	G/G	A/A	C9	51,374,427	C/C	G/G
C1	1,019,546	A/A	C/C	C2	209,455	C/C	A/A	C2	45,966,956	A/A	T/T	C3	27,037,929	C/C	T/T	C4	22,949,090	G/G	C/C	C5	8,080,343	G/G	C/C	C6	7,915,622	G/G	A/A	C7	21,584,178	A/A	C/C	C8	11,355,439	C/C	G/G	C9	2,715,019	A/A	T/T	C9	51,374,430	T/T	G/G
C1	1,019,628	A/A	C/C	C2	211,618	T/T	C/C	C2	45,967,534	A/A	T/T	C3	27,064,134	C/C	T/T	C4	22,949,156	T/T	C/C	C5	8,190,120	A/A	G/G	C6	7,915,737	C/C	A/A	C7	21,584,216	A/A	G/G	C8	11,373,466	T/T	A/A	C9	2,715,041	C/C	T/T	C9	51,374,673	G/G	A/A
C1	1,024,562	G/G	T/T	C2	211,640	T/T	G/G	C2	45,967,560	C/C	T/T	C3	27,064,335	C/C	T/T	C4	24,332,648	G/G	A/A	C5	8,190,197	C/C	T/T	C6	8,602,042	G/G	A/A	C7	21,763,923	T/T	A/A	C8	11,476,781	T/T	G/G	C9	2,717,066	T/T	C/C	C9	51,374,757	C/C	T/T
C1	1,024,565	T/T	C/C	C2	222,160	G/G	A/A	C2	45,971,962	T/T	C/C	C3	27,064,347	A/A	C/C	C4	24,339,619	A/A	T/T	C5	8,190,212	G/G	A/A	C6	8,646,860	C/C	T/T	C7	21,769,178	T/T	C/C	C8	11,572,536	G/G	T/T	C9	2,717,082	A/A	C/C	C9	51,394,053	A/A	G/G
C1	1,105,498	A/A	G/G	C2	222,300	A/A	G/G	C2	46,083,210	C/C	T/T	C3	27,075,276	A/A	T/T	C4	24,426,094	A/A	G/G	C5	8,276,629	G/G	C/C	C6	8,663,728	A/A	G/G	C7	21,769,215	G/G	T/T	C8	11,612,265	G/G	A/A	C9	2,727,677	G/G	A/A	C9	51,394,061	G/G	A/A
C1	1,105,553	C/C	T/T	C2	222,302	C/C	T/T	C2	46,120,971	C/C	T/T	C3	27,080,339	C/C	T/T	C4	24,426,134	A/A	G/G	C5	8,458,076	C/C	G/G	C6	8,663,188	A/A	T/T	C7	21,769,252	C/C	G/G	C8	11,612,412	C/C	T/T	C9	2,727,683	G/G	A/A	C9	51,394,152	C/C	T/T
C1	1,241,777	G/G	T/T	C2	241,356	T/T	A/A	C2	46,121,009	A/A	C/C	C3	27,219,022	T/T	A/A	C4	24,435,503	T/T	A/A	C5	8,492,967	A/A	C/C	C6	8,698,645	A/A	G/G	C7	21,769,268	G/G	A/A	C8	11,621,950	T/T	C/C	C9	2,787,348	G/G	A/A	C9	51,438,178	G/G	A/A
C1	1,242,106	T/T	G/G	C2	333,671	C/C	T/T	C2	46,121,026	G/G	T/T	C3	27,219,035	G/G	A/A	C4	24,459,524	T/T	C/C	C5	8,493,005	C/C	T/T	C6	8,756,897	C/C	A/A	C7	21,813,567	G/G	C/C	C8	11,621,969	A/A	G/G	C9	2,809,904	A/A	G/G	C9	51,459,288	G/G	T/T
C1	1,242,142	G/G	T/T	C2	333,845	G/G	C/C	C2	46,366,234	A/A	G/G	C3	27,220,391	G/G	T/T	C4	24,543,763	A/A	G/G	C5	8,646,257	T/T	A/A	C6	8,902,782	G/G	A/A	C7	21,813,652	G/G	T/T	C8	11,829,117	T/T	A/A	C9	2,809,920	A/A	G/G	C9	51,459,292	A/A	T/T
C1	1,242,469	A/A	G/G	C2	333,848	C/C	G/G	C2	46,366,302	G/G	A/A	C3	27,245,571	G/G	A/A	C4	24,845,675	T/T	A/A	C5	8,689,030	G/G	T/T	C6	9,197,004	G/G	C/C	C7	21,813,655	T/T	C/C	C8	11,918,426	A/A	G/G	C9	2,862,722	G/G	A/A	C9	51,470,109	A/A	G/G
C1	1,242,511	A/A	T/T	C2	395,705	A/A	T/T	C2	46,366,304	A/A	G/G	C3	27,245,587	T/T	A/A	C4	24,889,981	G/G	A/A	C5	8,689,045	A/A	C/C	C6	9,365,341	T/T	C/C	C7	21,934,240	G/G	C/C	C8	11,958,099	A/A	G/G	C9	2,862,730	T/T	C/C	C9	51,470,333	A/A	G/G
C1	1,369,909	T/T	A/A	C2	414,178	C/C	T/T	C2	46,827,854	T/T	C/C	C3	27,245,588	A/A	C/C	C4	25,466,901	A/A	C/C	C5	8,689,063	T/T	A/A	C6	9,400,626	T/T	G/G	C7	22,029,562	C/C	A/A	C8	11,958,134	C/C	T/T	C9	2,864,108	C/C	T/T	C9	51,471,084	C/C	T/T
C1	1,377,974	C/C	T/T	C2	414,187	C/C	A/A	C2	46,832,729	C/C	T/T	C3	27,375,701	T/T	C/C	C4	25,729,526	A/A	T/T	C5	8,689,069	C/C	T/T	C6	9,412,206	T/T	G/G	C7	22,043,769	T/T	C/C	C8	12,049,396	T/T	A/A	C9	2,902,039	A/A	G/G	C9	51,553,184	G/G	A/A
C1	1,377,993	A/A	G/G	C2	525,575	A/A	G/G	C2	46,876,629	A/A	G/G	C3	27,631,494	G/G	C/C	C4	25,747,401	T/T	G/G	C5	8,689,075	T/T	C/C	C6	9,695,628	A/A	T/T	C7	22,109,897	A/A	C/C	C8	12,091,503	T/T	C/C	C9	2,902,042	T/T	G/G	C9	51,715,857	C/C	T/T
C1	1,377,998	T/T	C/C	C2	527,050	T/T	A/A	C2	46,876,648	A/A	G/G	C3	27,707,729	A/A	C/C	C4	25,767,209	T/T	G/G	C5	8,767,070	A/A	C/C	C6	9,696,173	C/C	T/T	C7	22,109,897	T/T	C/C	C8	12,391,354	C/C	T/T	C9	2,902,129	G/G	T/T	C9	51,715,865	C/C	T/T
C1	1,378,051	T/T	G/G	C2	591,689	T/T	C/C	C2	46,922,541	G/G	C/C	C3	27,707,805	A/A	T/T	C4	26,171,845	T/T	A/A	C5	8,915,015	A/A	G/G	C6	9,696,186	G/G	T/T	C7	22,380,350	C/C	T/T	C8	12,403,211	G/G	C/C	C9	2,902,141	G/G	C/C	C9	51,791,042	A/A	G/G
C1	1,378,172	A/A	C/C	C2	591,759	G/G	C/C	C2	46,948,835	C/C	G/G	C3	27,707,816	T/T	C/C	C4	26,171,859	T/T	G/G	C5	8,993,002	T/T	C/C	C6	9,696,216	C/C	T/T	C7	22,438,663	G/G	C/C	C8	12,408,854	C/C	T/T	C9	2,920,600	A/A	T/T	C9	51,791,308	G/G	T/T
C1	1,425,040	C/C	A/A	C2	620,517	A/A	G/G	C2	46,998,180	G/G	A/A	C3	27,710,786	C/C	G/G	C4	26,171,863	T/T	G/G	C5	9,172,935	G/G	T/T	C6	9,699,354	T/T	A/A	C7	22,442,243	G/G	A/A	C8	12,933,948	C/C	T/T	C9	2,935,281	C/C	T/T	C9	51,803,073	T/T	G/G
C1	1,425,746	A/A	C/C	C2	666,637	C/C	A/A	C2	47,070,032	A/A	G/G	C3	27,712,353	G/G	A/A	C4	26,262,091	T/T	G/G	C5	9,183,604	T/T	A/A	C6	9,735,853	G/G	A/A	C7	22,474,103	C/C	A/A	C8	12,964,785	C/C	T/T	C9	3,069,453	C/C	T/T	C9	51,807,336	A/A	G/G
C1	1,443,929	C/C	T/T	C2	666,663	C/C	T/T	C2	47,128,814	T/T	G/G	C3	27,855,855	A/A	G/G	C4	26,354,042	T/T	C/C	C5	9,198,309	A/A	G/G	C6	9,737,283	C/C	G/G	C7	22,507,753	G/G	A/A	C8	12,964,787	G/G	A/A	C9	3,069,456	G/G	T/T	C9	51,835,212	C/C	A/A
C1	1,451,104	G/G	A/A	C2	675,361	A/A	C/C	C2	47,198,456	G/G	A/A	C3	27,866,482	G/G	T/T	C4	26,836,142	G/G	T/T	C5	9,199,497	T/T	A/A	C6	9,738,059	A/A	C/C	C7	22,514,576	G/G	A/A	C8	12,995,366	T/T	G/G	C9	3,071,058	G/G	T/T	C9	51,877,611	C/C	G/G
C1	1,649,764	T/T	A/A	C2	675,366	C/C	A/A	C2	47,387,515	A/A	G/G	C3	27,866,483	A/A	T/T	C4	26,868,826	T/T	G/G	C5	9,200,904	G/G	A/A	C6	9,738,177	A/A	G/G	C7	22,629,263	A/A	G/G	C8	14,131,801	G/G	A/A	C9	3,074,807	T/T	C/C	C9	51,981,299	T/T	C/C
C1	1,687,775	C/C	T/T	C2	675,383	T/T	C/C	C2	47,523,197	C/C	T/T	C3	27,887,330	A/A	G/G	C4	26,933,868	A/A	C/C	C5	9,327,284	A/A	C/C	C6	9,738,181	A/A	T/T	C7	22,629,269	G/G	A/A	C8	14,180,526	G/G	T/T	C9	3,084,399	C/C	A/A	C9	52,023,736	T/T	C/C
C1	1,689,170	C/C	T/T	C2	678,978	A/A	C/C	C2	47,523,372	G/G	T/T	C3	27,996,527	G/G	C/C	C4	26,933,993	A/A	G/G	C5	9,478,300	T/T	C/C	C6	9,788,551	G/G	A/A	C7	22,688,589	A/A	G/G	C8	14,312,018	A/A	G/G	C9	3,242,397	C/C	A/A	C9	52,023,739	A/A	G/G
C1	1,843,703	T/T	C/C	C2	694,385	T/T	G/G	C2	4																																		

Appendix III

Table S3: Cont. : Differences in Allelic variants at various chromosomes positions in parent lines; the cultivated rapid cycling DHSL150 and C07007.

Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007								
C1	2,093,928	A/A	G/G	C2	1,242,066	A/A	G/G	C2	49,043,008	T/T	A/A	C3	28,813,920	T/T	C/C	C4	29,074,559	T/T	C/C	C5	10,056,072	C/C	A/A	C6	13,237,487	G/G	A/A	C7	23,956,053	A/A	G/G	C8	16,542,210	G/G	T/T	C9	4,011,984	C/C	T/T	C9	52,838,300	G/G	A/A
C1	2,170,837	T/T	A/A	C2	1,264,601	T/T	G/G	C2	49,043,014	C/C	T/T	C3	28,813,992	G/G	C/C	C4	29,086,522	C/C	A/A	C5	10,061,383	T/T	C/C	C6	13,237,507	T/T	C/C	C7	23,956,146	A/A	C/C	C8	16,565,117	T/T	C/C	C9	4,042,682	T/T	C/C	C9	52,838,306	G/G	A/A
C1	2,222,532	T/T	A/A	C2	1,264,729	A/A	T/T	C2	49,043,026	A/A	G/G	C3	28,814,016	A/A	T/T	C4	29,086,535	A/A	T/T	C5	10,314,582	G/G	T/T	C6	13,237,617	T/T	G/G	C7	23,956,211	A/A	G/G	C8	16,565,139	C/C	T/T	C9	4,053,613	A/A	C/C	C9	52,858,882	T/T	C/C
C1	2,403,680	T/T	C/C	C2	1,294,768	A/A	G/G	C2	49,269,047	C/C	T/T	C3	28,940,402	C/C	T/T	C4	29,086,814	C/C	A/A	C5	10,314,632	A/A	G/G	C6	13,544,449	G/G	C/C	C7	23,956,312	A/A	G/G	C8	16,577,957	G/G	A/A	C9	4,167,609	T/T	C/C	C9	52,858,908	G/G	A/A
C1	2,405,495	T/T	G/G	C2	1,365,621	G/G	T/T	C2	49,269,087	C/C	T/T	C3	28,940,421	A/A	G/G	C4	29,382,037	A/A	G/G	C5	10,315,863	T/T	C/C	C6	13,701,398	G/G	A/A	C7	24,039,370	T/T	A/A	C8	16,580,540	A/A	T/T	C9	4,175,216	A/A	T/T	C9	52,864,989	C/C	A/A
C1	2,408,342	A/A	G/G	C2	1,413,049	C/C	T/T	C2	49,387,130	T/T	C/C	C3	28,962,590	A/A	G/G	C4	29,382,008	C/C	A/A	C5	10,444,571	T/T	A/A	C6	13,701,423	G/G	A/A	C7	24,165,204	G/G	A/A	C8	16,580,546	C/C	T/T	C9	4,193,337	T/T	C/C	C9	52,908,900	T/T	C/C
C1	2,412,136	T/T	C/C	C2	1,413,107	C/C	T/T	C2	49,698,532	C/C	T/T	C3	29,031,876	T/T	G/G	C4	29,602,230	G/G	C/C	C5	10,656,263	C/C	A/A	C6	13,716,495	A/A	G/G	C7	24,165,284	T/T	C/C	C8	16,580,588	C/C	T/T	C9	4,193,349	T/T	C/C	C9	52,908,966	C/C	T/T
C1	2,481,077	C/C	T/T	C2	1,413,142	T/T	G/G	C2	49,713,183	C/C	T/T	C3	29,031,882	A/A	G/G	C4	29,811,345	C/C	G/G	C5	10,890,797	A/A	C/C	C6	13,721,485	T/T	A/A	C7	24,269,738	C/C	T/T	C8	16,606,388	T/T	A/A	C9	4,193,385	A/A	G/G	C9	52,909,074	T/T	G/G
C1	2,489,091	T/T	G/G	C2	1,413,269	A/A	G/G	C2	49,713,303	C/C	T/T	C3	29,031,900	C/C	G/G	C4	29,814,424	G/G	A/A	C5	10,891,500	G/G	A/A	C6	13,864,586	A/A	T/T	C7	24,269,747	T/T	C/C	C8	16,618,699	T/T	C/C	C9	4,195,273	C/C	G/G	C9	52,914,226	C/C	T/T
C1	2,493,006	T/T	G/G	C2	1,413,551	T/T	C/C	C2	49,819,380	T/T	C/C	C3	29,031,925	G/G	C/C	C4	29,814,501	T/T	G/G	C5	11,070,077	C/C	A/A	C6	13,864,639	T/T	C/C	C7	24,269,763	C/C	T/T	C8	16,656,671	G/G	T/T	C9	4,226,933	C/C	G/G	C9	52,921,263	T/T	A/A
C1	2,546,530	T/T	G/G	C2	1,424,862	T/T	G/G	C2	49,819,503	C/C	G/G	C3	29,103,217	G/G	A/A	C4	29,814,504	C/C	A/A	C5	11,070,087	G/G	A/A	C6	14,017,162	G/G	A/A	C7	24,269,986	T/T	C/C	C8	16,676,275	G/G	G/G	C9	4,362,491	G/G	T/T	C9	52,931,124	C/C	T/T
C1	2,546,533	T/T	C/C	C2	1,424,892	G/G	T/T	C2	49,819,581	G/G	A/A	C3	29,105,463	C/C	T/T	C4	29,814,539	C/C	T/T	C5	11,070,126	A/A	C/C	C6	14,039,687	G/G	T/T	C7	24,292,702	G/G	A/A	C8	16,721,166	G/G	A/A	C9	4,362,492	G/G	A/A	C9	52,960,568	A/A	T/T
C1	2,586,742	T/T	C/C	C2	1,424,958	T/T	G/G	C2	49,821,295	A/A	G/G	C3	29,118,279	C/C	T/T	C4	29,937,496	C/C	T/T	C5	11,085,101	A/A	T/T	C6	14,039,734	C/C	T/T	C7	24,405,246	G/G	A/A	C8	16,721,175	C/C	T/T	C9	4,362,523	A/A	G/G	C9	52,960,592	T/T	C/C
C1	2,606,590	G/G	A/A	C2	1,433,637	G/G	A/A	C2	49,821,302	G/G	C/C	C3	29,118,312	C/C	A/A	C4	29,940,413	T/T	C/C	C5	11,114,829	A/A	C/C	C6	14,039,770	T/T	G/G	C7	24,405,314	C/C	T/T	C8	16,721,199	A/A	C/C	C9	4,362,618	C/C	T/T	C9	53,045,946	G/G	A/A
C1	2,635,717	G/G	A/A	C2	1,433,696	T/T	A/A	C2	50,026,194	A/A	T/T	C3	29,357,173	A/A	G/G	C4	29,940,881	A/A	G/G	C5	11,114,872	A/A	G/G	C6	14,039,810	T/T	A/A	C7	24,406,530	G/G	A/A	C8	16,721,295	T/T	A/A	C9	4,394,058	C/C	T/T	C9	53,045,961	A/A	G/G
C1	2,768,808	A/A	G/G	C2	1,466,773	T/T	C/C	C2	50,052,409	C/C	T/T	C3	29,516,511	C/C	A/A	C4	29,941,233	G/G	C/C	C5	11,119,557	C/C	G/G	C6	14,057,512	T/T	G/G	C7	24,406,545	G/G	C/C	C8	16,721,313	T/T	C/C	C9	4,394,112	T/T	A/A	C9	53,045,970	G/G	A/A
C1	2,768,830	G/G	C/C	C2	1,482,040	T/T	C/C	C2	50,136,949	A/A	G/G	C3	29,519,209	T/T	G/G	C4	29,941,335	G/G	T/T	C5	11,232,490	G/G	A/A	C6	14,331,313	G/G	A/A	C7	24,406,596	C/C	T/T	C8	16,731,984	G/G	C/C	C9	4,414,326	G/G	A/A	C9	53,045,991	G/G	A/A
C1	2,768,917	A/A	G/G	C2	1,658,484	A/A	G/G	C2	50,155,350	G/G	A/A	C3	29,519,210	T/T	C/C	C4	29,941,348	C/C	T/T	C5	11,265,390	C/C	G/G	C6	14,349,148	A/A	C/C	C7	24,406,607	T/T	C/C	C8	17,120,152	A/A	T/T	C9	4,486,897	A/A	T/T	C9	53,053,948	A/A	G/G
C1	2,810,286	A/A	G/G	C2	1,658,488	A/A	C/C	C2	50,626,560	A/A	G/A	C3	29,622,889	A/A	C/C	C4	29,962,909	C/C	G/G	C5	11,265,418	C/C	A/A	C6	14,382,218	A/A	G/G	C7	24,434,576	G/G	C/C	C8	17,120,153	T/T	C/C	C9	4,516,647	A/A	T/T	C9	53,053,963	A/A	T/T
C1	2,843,842	A/A	C/C	C2	1,658,509	G/G	A/A	C2	50,626,597	T/T	A/A	C3	29,626,185	A/A	G/G	C4	30,443,833	A/A	C/C	C5	11,271,114	C/C	A/A	C6	14,442,907	C/C	G/G	C7	24,434,590	A/A	G/G	C8	17,120,194	A/A	G/G	C9	4,518,618	T/T	G/G	C9	53,053,975	A/A	T/T
C1	2,843,854	A/A	G/G	C2	1,663,064	A/A	G/G	C2	50,626,609	T/T	C/C	C3	29,626,274	C/C	T/T	C4	30,463,455	A/A	C/C	C5	11,353,894	C/C	G/G	C6	14,445,232	A/A	C/C	C7	24,434,646	G/G	A/A	C8	17,120,214	T/T	G/G	C9	4,544,908	C/C	T/T	C9	53,056,449	G/G	T/T
C1	2,844,507	C/C	T/T	C2	1,689,350	A/A	T/T	C2	50,637,567	A/A	G/C	C3	29,626,285	C/C	G/G	C4	30,493,237	T/T	C/C	C5	11,358,213	T/T	C/C	C6	14,445,325	A/A	T/T	C7	24,434,663	T/T	C/C	C8	17,128,702	A/A	C/C	C9	4,582,152	C/C	T/T	C9	53,092,408	C/C	T/T
C1	2,844,510	T/T	A/A	C2	1,690,493	A/A	G/G	C2	50,964,534	T/T	G/G	C3	29,626,286	A/A	G/G	C4	30,497,710	A/A	T/T	C5	11,358,331	G/G	T/T	C6	14,446,995	A/A	C/C	C7	24,435,001	G/G	T/T	C8	17,128,705	A/A	T/T	C9	4,775,602	C/C	T/T	C9	53,092,468	T/T	C/C
C1	2,850,100	G/G	A/A	C2	1,690,496	G/G	A/A	C2	50,992,061	C/C	A/A	C3	29,627,356	T/T	C/C	C4	30,721,110	C/C	T/T	C5	11,377,618	G/G	T/T	C6	14,497,145	A/A	G/G	C7	24,435,626	C/C	T/T	C8	17,130,969	A/A	C/C	C9	4,775,633	G/G	A/A	C9	53,092,525	A/A	G/G
C1	2,850,103	A/A	C/C	C2	1,806,600	G/G	A/A	C2	50,992,088	G/G	A/A	C3	29,627,597	G/G	T/T	C4	30,721,119	G/G	A/A	C5	11,398,093	G/G	A/A	C6	14,497,159	G/G	T/T	C7	24,435,693	A/A	G/G	C8	17,142,807	T/T	A/A	C9	4,775,650	G/G	A/A	C9	53,092,615	C/C	T/T
C1	2,850,112	G/G	A/A	C2	1,806,627	T/T	A/A	C2	50,992,097	G/G	A/A	C3	29,737,774	G/G	C/C	C4	31,100,939	G/G	T/T	C5	11,398,794	T/T	C/C	C6	15,065,063	T/T	C/C	C7	24,436,052	A/A	G/G	C8	17,143,236	A/A	C/C	C9	4,942,541	A/A	T/T	C9	53,093,857	A/A	G/G
C1	2,901,543	G/G	A/A	C2	1,806,639	G/G	T/T	C2	50,994,702	T/T	C/C	C3	29,758,390	A/A	C/C	C4	31,201,340	G/G	A/A	C5	11,464,560	G/G	T/T	C6	15,065,685	A/A	G/G	C7	24,452,259	G/G	T/T	C8	17,270,614	G/G	C/C	C9	4,942,568	T/T	C/C	C9	53,093,863	G/G	T/T
C1	2,901,546	T/T	C/C	C2	1,806,713	C/C	T/T	C2	51,283,442	A/A	T/T	C3	29,860,956	T/T	C/C	C4	31,201,389	C/C	A/A	C5	11,483,266	A/A	G/G	C6	15,065,727	G/G	T/T	C7	24,452,365	T/T	C/C	C8	17,274,333	A/A	G/G	C9	4,942,573	A/A	G/G	C9	53,093,927	G/G	T/T
C1	2,914,998	A/A	G/G	C2	1,809,217	G/G	C/C	C2	51,791,861	T/T	C/C	C3	29,860,960	C/C	T/T	C4	31,210,370	C/C	T/T	C5	11,488,794	T/T	C/C	C6	15,103,208	T/T	G/G	C7	24,489,599	T/T	A/A	C8	17,360,429	C/C	T/T	C9	4,960,885	T/T	C/C	C9	53,242,482	C/C	T/T
C1	2,922,579	T/T	C/C	C2	1,809,293	A/A	T/T	C2	51,791,873	C/C	T/T	C3	30,128,971	C/C	T/T	C4	31,210,388	G/G	A/A	C5	11,510,564	C/C	T/T	C6	15,179,104	C/C	A/A	C7	24,489,709	G/G	T/T	C8	17,457,400	C/C	T/T	C9	4,960,917	T/T	A/A	C9	53,261,214	G/G	A/A
C1	2,922,599	A/A	G/G	C2	1,826,117	A/A	G/G	C2	51,885,119	T/T	C/C	C3	30,129,012	C/C	A/A	C4	31,387,303	T/T	C/C	C5	11,510,598	G/G	A/A	C6	15,217,365	T/T	C/C	C7	24,533,384	T/T	C/C	C8	17,489,150	T/T	G/G	C9	4,987,592	T/T	C/C	C9	53,271,723	T/T	A/A
C1	2,922,638	G/G	A/A	C2	2,002,305	G/G	T/T	C2	51,885,128	C/C	T/T	C3	30,129,072	C/C	T/T	C4	31,420,623	A/A	G/G	C5	11,831,999	G/G	A/A	C6	15,217,464	C/C	G/G	C7	24,533,391	G/G	C/C	C8	17,521,810	C/C	G/G	C9	4,999,655	G/G	A/A	C9	53,276,185	C/C	A/A
C1	2,926,663	G/G	A/A	C2	2,154,992	A/A	G/G	C2	51,885,212	G/G	A/A	C3	30,129,089	C/C	T/T	C4	31,432,394	G/G	A/A	C5	11,832,059	G/G	A/A	C6	15,218,298	A/A	G/G	C7	24,533														

Appendix III Cont.

Table S3: Cont. : Differences in Allelic variants at various chromosomes positions in parent lines; the cultivated rapid cycling DHSL150 and C07007.

Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007																
C1	3,369,163	A/A	G/G	C2	2,492,400	A/A	G/G	C2	2,492,875	G/G	A/A	C2	52,481,367	T/T	A/A	C3	31,328,556	T/T	C/C	C4	32,527,154	T/T	A/A	C5	12,389,883	C/C	T/T	C6	15,784,832	A/A	G/G	C7	25,566,917	A/A	G/G	C8	19,041,898	T/T	C/C	C9	5,383,695	G/G	T/T	C9	54,086,453	G/G	T/T
C1	3,371,471	A/A	G/G	C2	2,492,875	G/G	A/A	C2	52,481,367	T/T	A/A	C3	31,328,556	T/T	C/C	C4	32,527,167	C/C	T/T	C5	12,556,733	C/C	A/A	C6	15,887,579	A/A	G/G	C7	25,567,916	C/C	A/A	C8	19,051,305	A/A	C/C	C9	5,383,718	A/A	C/C	C9	54,086,540	A/A	G/G				
C1	3,374,485	T/T	C/C	C2	2,492,944	T/T	G/G	C2	52,481,522	A/A	T/T	C3	31,328,557	G/G	T/T	C4	32,527,283	G/G	A/A	C5	12,837,106	T/T	G/G	C6	15,910,688	G/G	C/C	C7	25,568,737	T/T	C/C	C8	19,053,944	C/C	T/T	C9	5,383,735	C/C	A/A	C9	54,086,574	A/A	T/T				
C1	3,416,632	G/G	T/T	C2	2,493,343	T/T	C/C	C2	52,680,644	A/A	T/T	C3	31,330,950	T/T	C/C	C4	32,527,304	T/T	C/C	C5	12,964,877	C/C	T/T	C6	15,940,094	T/T	G/G	C7	25,571,593	A/A	C/C	C8	19,054,022	G/G	A/A	C9	5,383,764	G/G	A/A	C9	54,086,583	A/A	G/G				
C1	3,416,824	C/C	G/G	C2	2,513,679	T/T	A/A	C2	52,680,661	G/G	A/A	C3	31,530,384	G/G	A/A	C4	32,527,326	C/C	G/G	C5	13,044,487	C/C	T/T	C6	16,421,712	T/T	C/C	C7	25,571,597	C/C	T/T	C8	19,054,037	A/A	G/G	C9	5,385,236	C/C	A/A	C9	54,388,973	G/G	T/T				
C1	3,419,658	T/T	C/C	C2	2,528,990	G/G	A/A	C2	52,684,073	T/T	A/A	C3	31,603,407	A/A	C/C	C4	32,527,830	C/C	G/G	C5	13,241,491	T/T	A/A	C6	16,422,776	T/T	A/A	C7	25,700,426	G/G	T/T	C8	19,054,514	A/A	G/G	C9	5,396,316	T/T	G/G	C9	54,539,892	T/T	C/C				
C1	3,448,840	C/C	G/G	C2	2,536,936	T/T	C/C	C3	37,890	G/G	T/T	C3	31,727,187	C/C	T/T	C4	32,527,855	A/A	G/G	C5	13,308,754	T/T	C/C	C6	16,803,664	T/T	A/A	C7	25,785,834	A/A	T/T	C8	19,061,669	C/C	T/T	C9	5,397,507	G/G	A/A								
C1	3,515,326	A/A	G/G	C2	2,556,346	A/A	G/G	C3	38,101	A/A	C/C	C3	31,743,829	G/G	C/C	C4	32,527,888	A/A	G/G	C5	13,521,800	C/C	T/T	C6	16,821,537	T/T	C/C	C7	25,934,897	T/T	G/G	C8	19,061,720	T/T	C/C	C9	5,399,323	T/T	G/G								
C1	3,593,740	T/T	C/C	C2	2,700,304	C/C	T/T	C3	90,348	G/G	T/T	C3	31,749,424	T/T	C/C	C4	32,527,900	G/G	T/T	C5	13,521,879	A/A	G/G	C6	16,821,652	A/A	C/C	C7	25,934,932	A/A	G/G	C8	19,061,734	A/A	C/C	C9	5,414,508	A/A	C/C								
C1	3,628,374	T/T	G/G	C2	2,700,308	T/T	C/C	C3	121,971	C/C	G/G	C3	31,749,473	C/C	T/T	C4	32,920,992	T/T	G/G	C5	13,522,306	A/A	G/G	C6	16,828,854	G/G	C/C	C7	25,962,484	G/G	A/A	C8	19,076,563	A/A	C/C	C9	5,454,300	T/T	C/C								
C1	3,628,386	G/G	C/C	C2	2,700,327	G/G	C/C	C3	176,098	C/C	A/A	C3	31,991,402	G/G	A/A	C4	32,955,076	G/G	A/A	C5	13,646,750	A/A	C/C	C6	16,900,661	G/G	T/T	C7	26,011,419	T/T	C/C	C8	19,076,606	C/C	T/T	C9	5,455,039	G/G	A/A								
C1	3,630,949	G/G	C/C	C2	2,700,349	G/G	A/A	C3	176,113	T/T	C/C	C3	32,021,781	G/G	A/A	C4	32,955,114	G/G	A/A	C5	13,822,410	A/A	G/G	C6	17,009,572	T/T	A/A	C7	26,021,270	A/A	G/G	C8	19,305,965	C/C	T/T	C9	5,471,520	G/G	T/T								
C1	3,630,982	A/A	C/C	C2	2,700,404	A/A	T/T	C3	183,268	A/A	C/C	C3	32,021,854	T/T	C/C	C4	33,319,240	A/A	C/C	C5	13,831,598	A/A	G/G	C6	17,009,598	A/A	C/C	C7	26,084,694	C/C	T/T	C8	19,305,981	A/A	G/G	C9	5,475,524	A/A	G/G								
C1	3,630,994	G/G	C/C	C2	2,710,096	T/T	C/C	C3	183,274	G/G	A/A	C3	32,249,119	C/C	T/T	C4	33,497,224	A/A	C/C	C5	13,831,608	A/A	C/C	C6	17,128,509	T/T	G/G	C7	26,084,725	C/C	A/A	C8	19,374,197	C/C	T/T	C9	5,475,547	G/G	A/A								
C1	3,632,540	T/T	C/C	C2	2,710,166	T/T	C/C	C3	189,983	A/A	G/G	C3	32,295,450	G/G	T/T	C4	33,843,314	T/T	C/C	C5	13,831,654	T/T	G/G	C6	17,183,202	T/T	C/C	C7	26,096,924	A/A	C/C	C8	19,374,389	G/G	T/T	C9	5,475,273	G/G	A/A								
C1	3,641,579	A/A	C/C	C2	2,864,268	T/T	A/A	C3	349,244	A/A	T/T	C3	32,295,517	C/C	A/A	C4	33,843,339	G/G	A/A	C5	13,831,694	T/T	C/C	C6	17,183,231	A/A	C/C	C7	26,113,208	G/G	T/T	C8	19,412,787	C/C	T/T	C9	5,475,274	G/G	A/A								
C1	3,678,779	C/C	G/G	C2	2,920,742	A/A	T/T	C3	349,246	C/C	G/G	C3	32,333,598	G/G	A/A	C4	33,844,259	T/T	C/C	C5	13,831,731	A/A	G/G	C6	17,204,243	T/T	C/C	C7	26,113,302	C/C	A/A	C8	19,412,815	A/A	G/G	C9	5,724,121	T/T	A/A								
C1	3,678,855	G/G	A/A	C2	2,944,064	T/T	C/C	C3	349,339	T/T	A/A	C3	32,379,347	C/C	T/T	C4	34,032,789	A/A	C/C	C5	13,992,354	G/G	A/A	C6	17,674,828	T/T	A/A	C7	26,113,311	T/T	C/C	C8	19,460,501	T/T	C/C	C9	5,724,130	T/T	A/A								
C1	3,678,896	C/C	G/G	C2	2,957,420	A/A	G/G	C3	592,497	G/G	A/A	C3	32,379,358	T/T	C/C	C4	34,035,280	T/T	C/C	C5	14,000,504	A/A	G/G	C6	17,808,658	A/A	T/T	C7	26,144,592	G/G	A/A	C8	19,690,835	T/T	C/C	C9	5,730,835	G/G	T/T								
C1	3,685,691	A/A	G/G	C2	3,008,042	T/T	C/C	C3	592,539	C/C	A/A	C3	32,379,410	A/A	G/G	C4	34,035,569	G/G	T/T	C5	14,058,294	C/C	T/T	C6	17,969,648	T/T	A/A	C7	26,436,410	G/G	C/C	C8	19,690,838	G/G	A/A	C9	5,730,887	C/C	T/T								
C1	3,685,742	G/G	C/C	C2	3,025,695	C/C	A/A	C3	596,508	G/G	T/T	C3	32,574,034	A/A	G/G	C4	34,112,474	T/T	G/G	C5	14,188,447	C/C	T/T	C6	18,259,772	T/T	A/A	C7	26,564,060	T/T	C/C	C8	19,690,893	T/T	C/C	C9	5,751,018	A/A	C/C								
C1	3,708,766	T/T	C/C	C2	3,036,849	G/G	A/A	C3	596,524	A/A	C/C	C3	32,583,943	T/T	G/G	C4	34,241,598	C/C	A/A	C5	14,236,677	T/T	C/C	C6	18,259,837	A/A	T/T	C7	26,573,455	G/G	A/A	C8	19,832,410	T/T	C/C	C9	5,754,077	T/T	C/C								
C1	3,708,787	A/A	G/G	C2	3,138,452	C/C	G/G	C3	596,533	T/T	C/C	C3	32,781,382	C/C	A/A	C4	34,241,659	A/A	G/G	C5	14,237,691	T/T	C/C	C6	18,260,801	A/A	G/G	C7	26,573,476	A/A	C/C	C8	19,841,624	A/A	T/T	C9	5,754,086	A/A	G/G								
C1	3,708,842	T/T	C/C	C2	3,182,010	A/A	T/T	C3	618,417	T/T	C/C	C3	33,775,207	T/T	A/A	C4	34,241,992	G/G	A/A	C5	14,307,161	T/T	C/C	C6	18,261,219	A/A	G/G	C7	26,597,787	A/A	G/G	C8	19,846,325	T/T	C/C	C9	5,754,089	T/T	C/C								
C1	3,731,201	C/C	T/T	C2	3,182,025	A/A	G/G	C3	618,550	C/C	T/T	C3	34,140,392	G/G	T/T	C4	34,596,615	G/G	C/C	C5	14,322,746	G/G	A/A	C6	18,261,222	A/A	G/G	C7	26,597,803	T/T	C/C	C8	19,846,382	T/T	A/A	C9	5,827,845	A/A	G/G								
C1	3,753,123	A/A	C/C	C2	3,182,082	G/G	A/A	C3	619,249	T/T	C/C	C3	34,215,544	T/T	C/C	C4	34,597,125	T/T	G/G	C5	14,325,237	T/T	C/C	C6	18,310,100	A/A	C/C	C7	26,598,947	C/C	A/A	C8	19,877,496	A/A	G/G	C9	5,827,876	C/C	T/T								
C1	3,771,854	A/A	G/G	C2	3,247,045	T/T	C/C	C3	696,684	G/G	A/A	C3	34,493,481	A/A	G/G	C4	34,597,659	C/C	G/G	C5	14,341,510	T/T	G/G	C6	18,394,650	C/C	T/T	C7	26,601,921	A/A	G/G	C8	19,887,421	C/C	G/G	C9	5,852,473	C/C	T/T								
C1	3,771,876	T/T	C/C	C2	3,247,048	G/G	A/A	C3	710,944	A/A	G/G	C3	34,625,236	T/T	C/C	C4	34,621,511	A/A	G/G	C5	14,342,477	G/G	A/A	C6	18,452,501	T/T	C/C	C7	26,602,031	C/C	T/T	C8	19,894,147	A/A	G/G	C9	5,852,484	G/G	A/A								
C1	3,771,944	G/G	A/A	C2	3,247,062	T/T	C/C	C3	713,572	C/C	A/A	C3	34,747,783	G/G	A/A	C4	34,631,498	G/G	C/C	C5	14,381,619	G/G	C/C	C6	18,881,212	C/C	T/T	C7	26,707,467	T/T	G/G	C8	20,270,060	G/G	A/A	C9	6,016,481	C/C	G/G								
C1	3,793,683	G/G	C/C	C2	3,315,602	C/C	A/A	C3	716,230	A/A	T/T	C3	34,781,226	G/G	T/T	C4	34,636,416	T/T	G/G	C5	14,381,634	T/T	C/C	C6	18,881,290	A/A	G/G	C7	26,707,481	T/T	G/G	C8	20,270,062	T/T	A/A	C9	6,017,706	T/T	G/G								
C1	3,793,692	A/A	G/G	C2	3,350,194	A/A	G/G	C3	718,252	C/C	T/T	C3	34,814,552	G/G	A/A	C4	34,636,442	T/T	A/A	C5	14,381,661	C/C	T/T	C6	18,882,441	C/C	T/T	C7	26,904,107	C/C	A/A	C8	20,270,093	G/G	A/A	C9	6,017,723	G/G	A/A								
C1	3,895,159	G/G	A/A	C2	3,632,729	T/T	C/C	C3	718,258	T/T	C/C	C3	34,849,956	T/T	C/C	C4	34,636,618	G/G	T/T	C5	14,479,218	A/A	C/C	C6	18,942,531	G/G	C/C	C7	27,125,008	G/G	A/A	C8	20,275,262	G/G	A/A	C9	6,017,757	A/A	T/T								
C1	3,943,809	G/G	A/A	C2	3,711,758	A/A	C/C	C3	736,686	T/T	C/C	C3	34,850,013	A/A	T/T	C4	34,695,068	C/A	C/C	C5	14,479,308	T/T	C/C	C6	18,946,934	A/A	T/T	C7	27,125,076	C/C	T/T	C8	20,275,304	T/T	A/A	C9	6,096,849	T/T	C/C								
C1	3,943,875	A/A	C/C	C2	3,711,838	T/T	A/A	C3	736,701	C/C	A/A	C3	34,856,708	T/T	C/C	C4	34,784,114	G/G	A/A	C5	14,479,312	C/C	T/T	C6	19,044,574	G/G	A/A	C7	27,128,343	C/C	T/T	C8	20,275,322	C/C	A/A	C9	6,181,407	T/T	A/A								
C1	3,943,893	C/C	T/T	C2	3,713,850	C/C	A/A	C3	796,927	A/A	C/C	C3	34,856,753	C/C	T/T	C4	34,845,728	T/T	C/C	C5	14,718,991	T/T	G/G	C6	19,069,291	C/C	A/A	C7	27,191,508	C/C	A/A	C8	20,335,523	A/A	T/T	C9	6,191,706	T/T	A/A								
C1	3,945,736	G/G	T/T	C2	3,740,811	A/A	T/T	C3	797,089	C/C	A/A	C3	34,857,905	C/C	T/T	C4	34,896,882	A/A	C/C	C5	14,981,776	C/C	A/A																								

Appendix III *Cont.***Table S3: *Cont.* : Differences in Allelic variants at various chromosomes positions in parent lines; the cultivated rapid cycling DHSL150 and C07007.**

Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007								
C1	4,815,480	C/C	G/G	C2	4,353,934	A/A	C/C	C3	1,112,767	A/A	C/C	C3	36,339,035	C/C	T/T	C4	36,849,431	T/T	G/G	C5	16,321,481	A/A	G/G	C6	19,898,103	T/T	C/C	C7	29,188,363	G/G	T/T	C8	21,082,509	A/A	T/T	C9	6,816,975	C/C	T/T
C1	5,007,775	T/T	A/A	C2	4,390,335	A/A	T/T	C3	1,116,402	C/C	A/A	C3	36,341,089	A/A	T/T	C4	36,945,731	T/T	A/A	C5	16,334,063	A/A	G/G	C6	19,942,337	A/A	C/C	C7	29,245,988	T/T	A/A	C8	21,082,594	G/G	A/A	C9	6,816,976	G/G	A/A
C1	5,022,102	C/C	T/T	C2	4,390,372	C/C	T/T	C3	1,116,441	C/C	T/T	C3	36,341,113	C/C	A/A	C4	36,945,734	C/C	T/T	C5	16,364,864	A/A	C/C	C6	19,990,186	A/A	C/C	C7	29,275,116	C/C	T/T	C8	21,091,542	G/G	A/A	C9	6,936,947	A/A	T/T
C1	5,032,189	A/A	G/G	C2	4,644,991	C/C	T/T	C3	1,116,444	A/A	T/T	C3	36,341,211	T/T	C/C	C4	37,028,716	C/C	G/G	C5	16,431,763	T/T	G/G	C6	19,990,282	G/G	A/A	C7	29,275,127	A/A	C/C	C8	21,094,847	C/C	G/G	C9	6,971,714	G/G	A/A
C1	5,038,790	C/C	G/G	C2	5,109,646	C/C	A/A	C3	1,116,528	G/G	T/T	C3	36,344,421	T/T	C/C	C4	37,030,939	A/A	G/G	C5	16,436,062	G/G	A/A	C6	19,996,490	T/T	A/A	C7	29,275,235	A/A	G/G	C8	21,131,388	C/C	T/T	C9	6,981,281	G/G	T/T
C1	5,038,976	G/G	A/A	C2	5,164,379	T/T	A/A	C3	1,116,690	A/A	T/T	C3	36,349,634	T/T	C/C	C4	37,142,926	G/G	A/A	C5	16,679,059	C/C	T/T	C6	19,996,533	T/T	C/C	C7	29,275,282	G/G	A/A	C8	21,131,404	A/A	C/C	C9	6,987,874	A/A	T/T
C1	5,038,982	T/T	C/C	C2	5,314,284	C/C	T/T	C3	1,128,764	C/C	T/T	C3	36,365,707	G/G	C/C	C4	37,142,991	T/T	A/A	C5	16,724,647	G/G	A/A	C6	19,996,848	A/A	G/C	C7	29,280,478	A/A	G/A	C8	21,133,174	G/G	A/A	C9	6,987,891	A/A	C/C
C1	5,268,004	G/G	T/T	C2	5,376,137	C/C	A/A	C3	1,218,265	C/C	T/T	C3	36,365,809	C/C	A/A	C4	37,191,600	T/T	C/C	C5	16,812,585	A/A	G/G	C6	20,081,331	C/C	A/A	C7	29,343,016	A/A	T/T	C8	21,141,203	A/A	G/G	C9	6,987,921	T/T	C/C
C1	6,095,543	T/T	A/A	C2	5,376,168	C/C	A/A	C3	1,224,759	G/G	A/A	C3	36,365,830	G/G	T/T	C4	37,287,201	A/A	C/C	C5	16,813,987	A/A	G/C	C6	20,081,355	A/A	G/C	C7	29,382,530	C/C	A/A	C8	21,272,241	C/C	T/T	C9	7,169,685	C/C	G/G
C1	6,362,796	G/G	T/T	C2	5,376,189	G/G	A/A	C3	1,292,415	T/T	C/C	C3	36,369,710	A/A	C/C	C4	37,311,378	T/T	C/C	C5	16,867,642	C/C	G/G	C6	20,081,374	T/T	A/A	C7	29,382,544	A/A	C/C	C8	21,288,696	G/G	T/T	C9	7,177,229	G/G	C/C
C1	6,362,950	T/T	G/G	C2	5,376,304	T/T	C/C	C3	1,300,969	C/C	T/T	C3	36,369,711	T/T	G/G	C4	37,346,268	T/T	C/C	C5	16,867,684	A/A	C/C	C6	20,438,635	A/A	G/G	C7	29,408,978	C/C	A/A	C8	21,329,260	A/A	T/T	C9	7,349,467	A/A	G/G
C1	6,370,637	A/A	G/G	C2	5,382,474	A/A	G/G	C3	1,339,226	T/T	C/C	C3	36,369,744	C/C	A/A	C4	37,346,292	T/T	A/A	C5	16,867,738	A/A	T/T	C6	20,447,573	G/G	A/A	C7	29,417,229	T/T	A/A	C8	21,329,312	C/C	T/T	C9	7,431,363	A/A	C/C
C1	6,411,382	C/C	T/T	C2	5,387,049	G/G	A/A	C3	1,367,517	C/C	T/T	C3	36,374,770	T/T	A/A	C4	37,351,502	G/G	A/A	C5	16,916,441	T/T	C/C	C6	21,485,542	G/G	T/T	C7	29,490,175	C/C	T/T	C8	21,370,893	G/G	A/A	C9	7,431,425	A/A	T/T
C1	6,457,359	G/G	A/A	C2	5,387,150	C/C	T/T	C3	1,367,518	C/C	A/A	C3	36,374,844	G/G	A/A	C4	37,359,615	A/A	T/T	C5	16,916,574	A/A	G/C	C6	21,671,923	T/T	C/C	C7	29,490,177	A/A	G/C	C8	21,373,840	A/A	T/T	C9	7,526,754	A/A	G/G
C1	6,625,276	C/C	T/T	C2	5,387,189	T/T	C/C	C3	1,378,588	A/A	G/G	C3	36,374,931	C/C	T/T	C4	37,359,616	T/T	C/C	C5	17,043,472	T/T	C/C	C6	21,675,183	G/G	A/A	C7	29,898,537	T/T	C/C	C8	21,374,031	A/A	T/T	C9	7,544,119	A/A	G/G
C1	6,638,654	T/T	A/A	C2	5,387,861	T/T	C/C	C3	1,378,614	A/A	G/G	C3	36,374,940	T/T	C/C	C4	37,359,636	A/A	G/G	C5	17,045,797	T/T	G/G	C6	22,114,323	G/G	A/A	C7	30,136,803	C/C	T/T	C8	21,439,231	A/A	G/G	C9	7,582,988	A/A	G/G
C1	6,668,386	C/C	T/T	C2	5,582,180	T/T	C/C	C3	1,378,633	A/A	G/G	C3	36,448,981	A/A	G/G	C4	37,617,302	T/T	G/G	C5	17,178,870	C/C	A/A	C6	22,155,812	C/C	T/T	C7	30,274,892	T/T	C/C	C8	21,449,086	G/G	T/T	C9	7,583,072	T/T	C/C
C1	6,764,781	T/T	C/C	C2	5,609,425	A/A	C/C	C3	1,476,791	C/C	T/T	C3	36,453,781	A/A	C/C	C4	37,617,309	C/C	A/A	C5	17,268,098	C/C	T/T	C6	22,399,168	G/G	C/C	C7	30,274,913	A/A	G/C	C8	21,449,130	C/C	G/G	C9	7,583,080	A/A	C/C
C1	6,898,088	C/C	G/G	C2	5,609,924	A/A	C/C	C3	1,476,861	G/G	A/A	C3	36,453,783	C/C	A/A	C4	37,617,641	A/A	G/G	C5	17,294,314	T/T	C/C	C6	22,399,201	T/T	C/C	C7	30,276,323	G/G	A/A	C8	21,484,835	A/A	C/C	C9	7,584,623	C/C	G/G
C1	6,949,867	A/A	T/T	C2	5,610,026	G/G	T/T	C3	1,476,865	A/A	G/G	C3	36,457,990	T/T	C/C	C4	37,618,659	C/C	T/T	C6	22,434,009	A/A	G/G	C7	30,441,581	A/A	G/G	C8	21,488,498	C/C	A/A	C9	7,585,554	C/C	T/T				
C1	6,966,753	A/A	C/C	C2	5,631,364	A/A	T/T	C3	1,479,607	T/T	C/C	C3	36,514,510	G/G	A/A	C4	37,618,662	C/C	T/T	C5	17,298,371	A/A	G/G	C6	22,434,161	C/C	T/T	C7	30,441,596	C/C	T/T	C8	21,488,518	C/C	A/A	C9	7,591,771	A/A	G/G
C1	6,966,784	A/A	G/G	C2	5,651,338	A/A	C/C	C3	1,505,807	T/T	G/G	C3	36,514,593	G/G	T/T	C4	37,618,701	T/T	C/C	C5	17,299,087	A/A	T/T	C6	22,434,185	T/T	C/C	C7	30,441,598	G/G	A/A	C8	21,493,521	G/G	T/T	C9	7,591,939	T/T	C/C
C1	6,966,793	C/C	T/T	C2	5,651,351	T/T	A/A	C3	1,613,259	A/A	T/T	C3	36,570,496	A/A	G/G	C4	37,906,088	A/A	G/G	C5	17,496,922	C/C	T/T	C6	22,434,219	T/T	C/C	C7	30,441,800	G/G	A/A	C8	21,503,348	T/T	C/C	C9	7,609,136	T/T	C/C
C1	6,966,842	G/G	C/C	C2	5,651,368	C/C	T/T	C3	1,653,434	T/T	C/C	C3	36,600,570	C/C	T/T	C4	37,906,094	G/G	A/A	C5	17,525,657	T/T	C/C	C6	22,434,233	G/G	A/A	C7	30,441,926	G/G	A/A	C8	21,573,897	C/C	T/T	C9	7,664,652	G/G	A/A
C1	6,966,861	G/G	A/A	C2	5,671,103	G/G	A/A	C3	1,678,096	A/A	G/G	C3	36,600,591	C/C	T/T	C4	37,906,220	C/C	G/G	C5	17,525,669	T/T	C/C	C6	22,564,381	G/G	T/T	C7	30,520,133	T/T	A/A	C8	21,610,152	C/C	T/T	C9	7,664,654	A/A	T/T
C1	6,967,665	G/G	A/A	C2	5,674,776	C/C	T/T	C3	1,678,110	C/C	T/T	C3	36,600,624	A/A	G/G	C4	38,112,542	C/C	G/G	C5	17,527,946	C/C	T/T	C6	22,660,090	G/G	T/T	C7	30,520,162	C/C	T/T	C8	21,778,000	T/T	C/C	C9	7,689,717	G/G	A/A
C1	6,967,756	A/A	G/G	C2	5,674,838	A/A	G/G	C3	1,721,286	A/A	C/C	C3	36,620,993	T/T	C/C	C4	38,112,561	T/T	C/C	C5	17,974,378	G/G	A/A	C6	23,003,328	C/C	T/T	C7	30,581,959	A/A	C/C	C8	21,778,036	C/C	T/T	C9	7,689,723	C/C	G/G
C1	7,156,481	T/T	A/A	C2	5,675,030	T/T	A/A	C3	1,869,050	T/T	C/C	C3	36,621,067	A/A	G/G	C4	38,139,174	G/G	A/A	C5	18,057,880	T/T	G/G	C6	23,256,321	A/A	T/T	C7	30,581,962	A/A	T/T	C8	21,885,500	G/G	A/A	C9	7,703,279	C/C	T/T
C1	7,206,307	C/C	T/T	C2	5,675,057	G/G	A/A	C3	1,869,067	C/C	G/G	C3	36,621,070	G/G	A/A	C4	38,139,222	T/T	C/C	C5	18,100,492	T/T	C/C	C6	23,256,387	A/A	G/G	C7	30,672,875	C/C	G/G	C8	21,885,636	G/G	A/A	C9	7,761,360	G/G	A/A
C1	7,206,319	G/G	A/A	C2	5,677,746	T/T	C/C	C3	1,879,783	C/C	A/A	C3	36,765,984	T/T	A/A	C4	38,148,977	G/G	A/A	C5	18,113,726	T/T	C/C	C6	23,301,501	A/A	T/T	C7	30,837,359	T/T	G/G	C8	21,885,663	G/G	C/C	C9	7,785,566	A/A	T/T
C1	7,235,221	A/A	G/G	C2	5,677,749	A/A	G/G	C3	1,890,867	G/G	A/A	C3	36,786,929	A/A	G/G	C4	38,150,634	T/T	G/G	C5	18,113,835	A/A	G/G	C6	23,301,534	G/G	A/A	C7	30,857,030	C/C	T/T	C8	21,923,375	T/T	G/G	C9	7,789,696	G/G	T/T
C1	7,347,598	C/C	T/T	C2	5,677,815	C/C	T/T	C3	1,912,212	C/C	T/T	C3	36,951,534	C/C	A/A	C4	38,178,375	G/G	A/A	C5	18,407,227	C/C	T/T	C6	23,301,608	C/C	T/T	C7	30,857,055	T/T	C/C	C8	22,246,472	A/A	C/C	C9	7,789,709	C/C	G/G
C1	7,746,104	A/A	G/G	C2	5,678,197	A/A	T/T	C3	1,912,300	C/C	T/T	C3	37,017,128	G/G	A/A	C4	38,178,387	T/T	C/C	C5	18,407,250	C/C	A/A	C6	23,480,529	C/C	T/T	C7	30,857,076	A/A	C/C	C8	22,246,473	C/C	G/G	C9	7,895,462	A/A	G/G
C1	7,746,117	A/A	C/C	C2	5,678,205	A/A	G/G	C3	1,912,381	A/A	G/G	C3	37,203,698	T/T	C/C	C4	38,196,664	C/C	G/G	C5	18,414,665	C/C	T/T	C6	23,680,951	G/G	A/A	C7	31,090,820	G/G	A/A	C8	22,420,903	C/C	T/T	C9	7,895,480	A/A	G/G
C1	7,746,149	T/T	A/A	C2	5,700,469	G/G	A/A	C3	1,921,765	A/A	T/T	C3	37,210,784	T/T	C/C	C4	38,214,763	C/C	G/G	C5	18,668,553	T/T	G/G	C6	23,680,960	G/G	A/A	C7	31,090,889	A/A	G/G	C8	22,452,435	T/T	C/C	C9	7,896,617	C/C	T/T
C1	7,766,784	C/C	G/G	C2	5,708,372	C/C	T/T	C3	1,921,770	C/C	T/T	C3	37,761,660	A/A	T/T	C4	38,245,688	T/T	C/C	C5	18,827,177	C/C	T/T	C6	23,749,324	C/C	T/T	C7	31,111,968	G/G	A/A	C8	22,713,805	C/C	T/T	C9	8,014,701	C/C	A/A
C1	7,770,676	A/A	G/G	C2	5,708,423	A/A	G/G																																

Appendix III *Cont.***Table S3: *Cont.* : Differences in Allelic variants at various chromosomes positions in parent lines; the cultivated rapid cycling DHSL150 and C07007.**

Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007								
C1	8,276,291	T/T	A/A	C2	6,307,157	C/C	G/G	C3	2,508,553	C/C	T/T	C3	38,653,270	C/C	A/A	C4	39,493,276	G/G	C/C	C5	20,525,285	T/T	C/C	C6	24,953,272	A/A	T/T	C7	32,086,282	T/T	C/C	C8	23,202,829	G/G	C/C	C9	8,413,641	C/C	A/A
C1	8,325,018	C/C	T/T	C2	6,307,175	G/G	A/A	C3	2,508,597	G/G	C/C	C3	38,724,247	T/T	G/G	C4	39,500,015	T/T	G/G	C5	20,600,760	G/G	T/T	C6	24,953,278	C/C	G/G	C7	32,314,714	G/G	T/T	C8	23,343,842	G/G	A/A	C9	8,413,650	C/C	A/A
C1	8,325,899	T/T	G/G	C2	6,354,799	A/A	G/G	C3	2,508,636	C/C	T/T	C3	38,854,277	G/G	A/A	C4	39,622,918	C/C	T/T	C5	20,711,078	C/C	T/T	C6	25,203,138	T/T	C/C	C7	32,439,174	C/C	T/T	C8	23,396,601	C/C	A/A	C9	8,413,723	C/C	T/T
C1	8,354,702	G/G	A/A	C2	6,645,814	T/T	C/C	C3	2,535,107	T/T	G/G	C3	38,899,899	C/C	T/T	C4	39,622,949	C/C	T/T	C5	20,711,247	G/G	A/A	C6	25,203,251	G/G	A/A	C7	32,462,135	G/G	T/T	C8	23,396,614	C/C	T/T	C9	8,414,098	C/C	T/T
C1	8,357,698	G/G	C/C	C2	6,682,898	G/G	T/T	C3	2,584,834	A/A	G/G	C3	38,989,345	A/A	C/C	C4	39,697,213	T/T	C/C	C5	20,711,265	A/A	G/G	C6	25,297,982	G/G	A/A	C7	32,462,363	G/G	T/T	C8	23,396,743	T/T	C/C	C9	8,438,751	G/G	A/A
C1	8,357,757	T/T	C/C	C2	6,943,932	A/A	G/G	C3	2,749,293	C/C	T/T	C3	38,992,716	C/C	A/A	C4	39,724,286	A/A	T/T	C5	20,711,308	C/C	T/T	C6	25,559,336	A/A	G/G	C7	32,462,405	A/A	G/G	C8	23,407,198	G/G	T/T	C9	8,467,580	A/A	G/G
C1	8,357,784	G/G	A/A	C2	6,991,997	C/C	A/A	C3	2,776,112	A/A	G/G	C3	39,037,597	T/T	C/C	C4	39,724,301	T/T	C/C	C5	20,758,302	A/A	C/C	C6	25,559,368	C/C	T/T	C7	32,478,522	A/A	G/G	C8	23,697,751	G/G	A/A	C9	8,656,283	G/G	T/T
C1	8,357,849	C/C	T/T	C2	7,029,891	T/T	G/G	C3	2,776,116	T/T	G/G	C3	39,215,391	G/G	T/T	C4	39,724,319	C/C	T/T	C5	20,852,303	C/C	T/T	C6	25,619,782	A/A	G/G	C7	32,478,536	G/G	A/A	C8	23,749,076	A/A	G/G	C9	8,656,351	C/C	T/T
C1	8,357,863	A/A	C/C	C2	7,034,262	A/A	G/G	C3	2,866,116	T/T	A/A	C3	39,215,483	G/G	A/A	C4	39,737,798	G/G	C/C	C5	21,109,741	C/C	T/T	C6	25,621,268	C/C	A/A	C7	32,495,007	C/C	T/T	C8	23,779,869	A/A	C/C	C9	8,682,525	A/A	C/C
C1	8,402,822	A/A	G/G	C2	7,037,297	A/A	G/G	C3	2,901,748	T/T	G/G	C3	39,253,822	C/C	T/T	C4	39,827,816	T/T	C/C	C5	21,109,758	T/T	C/C	C6	25,621,352	A/A	G/G	C7	32,622,853	T/T	C/C	C8	23,947,330	G/G	C/C	C9	8,809,878	T/T	A/A
C1	8,430,215	A/A	T/T	C2	7,111,971	T/T	G/G	C3	2,901,762	A/A	G/G	C3	39,689,198	T/T	C/C	C4	39,836,018	A/A	C/C	C5	21,166,445	G/G	A/A	C6	25,734,541	C/C	T/T	C7	32,724,209	T/T	C/C	C8	23,948,655	C/C	G/G	C9	8,881,393	T/T	A/A
C1	8,430,252	C/C	A/A	C2	7,111,978	A/A	C/C	C3	2,901,791	T/T	C/C	C3	39,689,219	G/G	T/T	C4	39,836,036	G/G	A/A	C5	21,480,742	G/G	A/A	C6	25,734,573	G/G	A/A	C7	32,813,208	T/T	A/A	C8	23,952,322	G/G	T/T	C9	8,947,111	C/C	T/T
C1	8,805,925	G/G	A/A	C2	7,142,691	A/A	G/G	C3	3,036,810	G/G	T/T	C3	39,700,909	G/G	A/A	C4	39,920,355	G/G	C/C	C5	21,480,765	T/T	C/C	C6	25,845,651	G/G	A/A	C7	32,857,129	G/G	T/T	C8	23,952,396	T/T	C/C	C9	8,986,159	A/A	C/C
C1	8,805,928	A/A	C/C	C2	7,142,706	T/T	G/G	C3	3,108,506	A/A	T/T	C3	39,765,920	C/C	T/T	C4	39,920,430	G/G	A/A	C5	21,480,887	C/C	T/T	C6	25,913,452	C/C	A/A	C7	32,897,383	T/T	C/C	C8	23,952,425	T/T	A/A	C9	8,986,258	T/T	A/A
C1	8,806,036	G/G	A/A	C2	7,185,236	T/T	G/G	C3	3,108,507	T/T	C/C	C3	39,979,322	T/T	G/G	C4	39,920,442	A/A	G/G	C5	21,680,209	A/A	G/G	C6	25,913,500	C/C	T/T	C7	32,983,191	C/C	A/A	C8	23,952,434	T/T	G/G	C9	9,036,890	A/A	C/C
C1	9,051,366	T/T	A/A	C2	7,325,272	G/G	A/A	C3	3,108,660	A/A	G/G	C3	39,982,132	C/C	A/A	C4	39,920,446	C/C	A/A	C5	21,690,070	A/A	G/G	C6	25,942,688	T/T	C/C	C7	32,984,233	A/A	G/G	C8	24,041,870	A/A	G/G	C9	9,038,429	T/T	G/G
C1	9,051,370	G/G	A/A	C2	7,394,860	G/G	A/A	C3	3,142,209	G/G	A/A	C3	40,188,713	C/C	A/A	C4	39,968,362	T/T	G/G	C5	21,723,125	T/T	C/C	C6	25,942,694	T/T	C/C	C7	33,103,836	T/T	C/C	C8	24,076,495	A/A	C/C	C9	9,135,006	A/A	C/C
C1	9,051,378	A/A	G/G	C2	7,397,729	G/G	A/A	C3	3,231,392	G/G	C/C	C3	40,188,851	T/T	C/C	C4	39,996,501	A/A	G/G	C5	21,794,108	T/T	G/G	C6	25,942,718	C/C	T/T	C7	33,104,610	C/C	T/T	C8	24,338,978	T/T	C/C	C9	9,142,018	G/G	A/A
C1	9,053,761	T/T	G/G	C2	7,442,970	G/G	C/C	C3	3,231,413	A/A	C/C	C3	40,225,157	G/G	T/T	C4	40,014,268	T/T	G/G	C5	21,794,592	C/C	A/A	C6	25,942,766	G/G	A/A	C7	33,324,524	G/G	C/C	C8	24,361,256	C/C	T/T	C9	9,142,046	C/C	A/A
C1	9,053,889	C/C	T/T	C2	7,462,630	T/T	C/C	C3	3,231,491	G/G	A/A	C4	40,261,392	G/G	A/A	C4	40,080,879	G/G	C/C	C5	21,828,167	C/C	T/T	C6	25,942,835	G/G	A/A	C7	33,324,551	C/C	T/T	C8	24,546,926	A/A	C/C	C9	9,259,729	G/G	T/T
C1	9,053,890	T/T	C/C	C2	7,484,182	G/G	A/A	C3	3,258,678	G/G	A/A	C3	40,501,649	C/C	T/T	C4	40,246,654	C/C	T/T	C5	21,833,010	C/C	A/A	C6	25,942,847	A/A	G/G	C7	33,324,563	C/C	T/T	C8	24,630,058	G/G	T/T	C9	9,286,159	G/G	T/T
C1	9,053,922	A/A	T/T	C2	7,697,735	A/A	G/G	C3	3,259,540	A/A	G/G	C3	40,501,724	G/G	A/A	C4	40,246,794	C/C	T/T	C5	21,865,865	C/C	G/G	C6	25,942,859	G/G	A/A	C7	33,324,611	A/A	G/G	C8	24,630,078	A/A	T/T	C9	9,286,524	T/T	A/A
C1	9,243,047	A/A	T/T	C2	7,743,065	C/C	T/T	C3	3,284,600	G/G	A/A	C3	40,502,858	A/A	G/G	C4	40,246,863	G/G	A/A	C5	21,933,165	G/G	T/T	C6	25,942,925	T/T	C/C	C7	33,324,629	T/T	C/C	C8	24,661,658	A/A	T/T	C9	9,287,057	A/A	C/C
C1	9,245,759	C/C	T/T	C2	7,836,440	C/C	G/G	C3	3,284,609	G/G	A/A	C3	40,572,119	C/C	T/T	C4	40,247,863	C/C	A/A	C5	21,970,755	T/T	A/A	C6	25,944,556	G/G	A/A	C7	33,324,671	A/A	T/T	C8	24,700,411	G/G	C/C	C9	9,305,165	G/G	A/A
C1	9,365,603	C/C	T/T	C2	7,896,154	T/T	C/C	C3	3,302,744	C/C	T/T	C3	40,574,714	G/G	T/T	C4	40,247,922	G/G	A/A	C5	21,970,839	T/T	C/C	C6	25,944,569	G/G	T/T	C7	33,325,592	C/C	T/T	C8	24,700,608	T/T	C/C	C9	9,869,237	A/A	T/T
C1	9,365,619	G/G	T/T	C2	8,356,028	C/C	T/T	C3	3,307,602	A/A	T/T	C3	40,680,214	G/G	A/A	C4	40,248,510	A/A	G/G	C5	21,970,947	G/G	A/A	C6	25,944,576	A/A	G/G	C7	33,325,619	T/T	C/C	C8	24,700,697	A/A	T/T	C9	9,869,274	T/T	G/G
C1	9,421,413	G/G	A/A	C2	8,476,668	T/T	A/A	C3	3,310,316	C/C	T/T	C3	40,690,091	A/A	G/G	C4	40,248,537	G/G	C/C	C5	21,970,970	G/G	T/T	C6	25,948,368	G/G	A/A	C7	33,350,917	G/G	A/A	C8	24,701,164	C/C	T/T	C9	9,917,684	G/G	T/T
C1	9,488,744	G/G	C/C	C2	8,476,812	T/T	A/A	C3	3,436,255	A/A	T/T	C3	40,752,989	T/T	C/C	C4	40,248,588	C/C	T/T	C5	21,996,158	C/C	T/T	C6	25,997,579	A/A	G/G	C7	33,350,939	C/C	G/G	C8	24,701,178	C/C	T/T	C9	10,014,761	T/T	C/C
C1	9,488,765	G/G	A/A	C2	8,510,105	A/A	G/G	C3	3,436,285	G/G	T/T	C3	40,995,036	T/T	G/G	C4	40,249,957	G/G	A/A	C5	22,032,828	T/T	G/G	C6	25,997,596	T/T	A/A	C7	33,350,947	G/G	A/A	C8	25,168,121	T/T	C/C	C9	10,015,913	A/A	G/G
C1	9,489,962	C/C	T/T	C2	8,520,975	G/G	T/T	C3	3,436,380	T/T	G/G	C3	41,060,098	C/C	G/G	C4	40,249,969	C/C	T/T	C5	22,077,614	T/T	C/C	C6	26,039,172	C/C	T/T	C7	33,351,022	A/A	C/C	C8	25,168,183	G/G	T/T	C9	10,020,479	A/A	G/G
C1	9,490,001	A/A	C/C	C2	8,521,071	T/T	C/C	C3	3,585,288	T/T	G/G	C3	41,288,466	T/T	G/G	C4	40,251,289	A/A	T/T	C5	22,373,727	C/C	T/T	C6	26,047,238	G/G	A/A	C7	33,351,028	G/G	A/A	C8	25,451,545	G/G	T/T	C9	10,569,640	T/T	G/G
C1	9,511,764	T/T	C/C	C2	8,540,048	T/T	C/C	C3	3,585,758	A/A	G/G	C3	41,341,458	G/G	C/C	C4	40,251,409	T/T	C/C	C5	22,562,072	A/A	G/G	C6	26,047,319	A/A	G/G	C7	33,351,037	G/G	C/C	C8	25,451,835	T/T	C/C	C9	10,569,737	C/C	A/A
C1	9,511,838	A/A	T/T	C2	8,581,072	G/G	A/A	C3	3,585,787	T/T	A/A	C3	41,450,761	A/A	T/T	C4	40,251,415	G/G	A/A	C5	22,562,075	T/T	A/A	C6	26,047,355	T/T	C/C	C7	33,692,553	A/A	G/G	C8	25,555,504	C/C	T/T	C9	10,569,794	A/A	T/T
C1	9,511,852	C/C	T/T	C2	8,581,088	T/T	G/G	C3	3,701,247	G/G	A/A	C3	41,557,925	C/C	A/A	C4	40,251,460	T/T	C/C	C5	22,562,102	C/C	G/G	C6	26,272,672	T/T	G/G	C7	33,692,573	G/G	T/T	C8	25,691,053	C/C	T/T	C9	10,605,563	T/T	C/C
C1	9,522,073	G/G	A/A	C2	8,601,975	G/G	T/T	C3	3,770,224	T/T	C/C	C3	41,685,359	A/A	C/C	C4	40,251,934	A/A	T/T	C5	22,562,213	C/C	G/G	C6	26,461,872	G/G	A/A	C7	33,894,835	A/A	G/G	C8	25,897,597	C/C	A/A	C9	10,605,631	G/G	A/A
C1	9,524,397	A/A	G/G	C2	8,612,789	C/C	A/A	C3	3,963,328	T/T	A/A	C3	41,853,601	A/A	G/G	C4	40,252,273	A/A	G/G	C5	22,564,770	C/C	T/T	C6	26,461,940	A/A	G/G	C7	33,915,110	C/C	G/G	C8	25,897,617	C/C	A/A	C9	10,612,908	C/C	T/T
C1	9,524,412																																						

Appendix III *Cont.***Table S3: *Cont.* : Differences in Allelic variants at various chromosomes positions in parent lines; the cultivated rapid cycling DHSL150 and C07007.**

Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007								
C1	10,769,894	A/A	T/T	C2	9,246,890	C/C	A/A	C3	4,596,108	A/A	G/G	C3	42,937,428	A/A	G/G	C4	40,874,070	A/A	G/G	C5	22,960,109	T/T	C/C	C6	27,078,828	G/G	C/C	C7	34,395,218	G/G	T/T	C8	27,303,524	G/G	A/A	C9	11,049,290	T/T	G/G
C1	10,771,008	A/A	T/T	C2	9,249,508	T/T	C/C	C3	4,633,791	T/T	A/A	C3	42,937,442	A/A	G/G	C4	40,877,853	C/C	T/T	C5	22,972,288	A/A	G/G	C6	27,078,911	A/A	T/T	C7	34,395,224	T/T	C/C	C8	27,336,743	A/A	G/G	C9	11,049,304	T/T	A/A
C1	10,859,686	G/G	C/C	C2	9,297,637	G/G	T/T	C3	4,636,473	A/A	G/G	C3	43,000,129	A/A	C/C	C4	41,637,553	A/A	G/G	C5	22,978,933	T/T	C/C	C6	27,081,539	G/G	A/A	C7	34,453,242	G/G	T/T	C8	27,338,031	A/A	C/C	C9	11,161,087	A/A	T/T
C1	10,860,960	T/T	A/A	C2	9,361,694	G/G	C/C	C3	4,671,554	A/A	T/T	C3	43,005,350	G/G	C/C	C4	41,707,163	T/T	C/C	C5	22,978,968	A/A	G/G	C6	27,126,094	T/T	C/C	C7	34,468,205	G/G	C/C	C8	27,426,217	T/T	A/A	C9	11,252,846	A/A	T/T
C1	11,053,637	C/C	T/T	C2	9,364,014	T/T	C/C	C3	4,708,418	A/A	G/G	C3	43,131,012	A/A	G/G	C4	41,816,429	T/T	C/C	C5	23,120,852	A/A	G/G	C6	27,522,256	G/G	A/A	C7	34,534,964	A/A	C/C	C8	27,447,484	C/C	T/T	C9	11,485,399	A/A	C/C
C1	11,053,817	C/C	A/A	C2	9,364,051	C/C	T/T	C3	4,713,570	T/T	A/A	C3	43,131,045	C/C	T/T	C4	41,816,471	A/A	C/C	C5	23,120,862	C/C	T/T	C6	27,522,478	T/T	C/C	C7	34,583,443	G/G	A/A	C8	27,697,505	A/A	T/T	C9	11,499,383	A/A	C/C
C1	11,101,901	G/G	A/A	C2	9,371,718	C/C	T/T	C3	4,713,612	G/G	T/T	C3	43,187,611	C/C	G/G	C4	41,816,477	A/A	G/G	C5	23,205,916	G/G	A/A	C6	27,710,116	A/A	G/G	C7	34,590,270	A/A	G/G	C8	27,704,251	T/T	C/C	C9	11,499,421	A/A	C/C
C1	11,214,670	G/G	A/A	C2	9,391,994	A/A	G/G	C3	4,731,076	A/A	G/G	C3	43,187,621	T/T	C/C	C4	41,839,863	T/T	C/C	C5	23,273,201	G/G	T/T	C6	27,710,155	G/G	A/A	C7	34,632,728	T/T	A/A	C8	27,736,106	C/C	G/G	C9	11,499,425	T/T	G/G
C1	11,216,197	T/T	G/G	C2	9,446,434	T/T	C/C	C3	4,750,998	T/T	C/C	C3	43,256,290	A/A	C/C	C4	42,015,238	A/A	G/G	C5	23,274,315	G/G	A/A	C6	27,727,668	C/C	G/G	C7	34,632,742	G/G	A/A	C8	27,739,594	C/C	T/T	C9	11,514,474	C/C	T/T
C1	11,216,323	G/G	A/A	C2	9,933,345	T/T	C/C	C3	4,766,337	C/C	A/A	C3	43,402,178	T/T	C/C	C4	42,053,933	A/A	G/G	C5	23,355,201	T/T	C/C	C6	27,904,406	G/G	A/A	C7	34,636,897	C/C	T/T	C8	27,747,355	A/A	G/G	C9	11,514,568	A/A	G/G
C1	11,238,171	T/T	C/C	C2	10,079,709	T/T	C/C	C3	4,766,395	G/G	C/C	C3	43,474,913	G/G	A/A	C4	42,053,984	A/A	G/G	C5	23,438,994	T/T	C/C	C6	27,904,449	A/A	G/G	C7	34,636,900	T/T	C/C	C8	27,781,499	A/A	T/T	C9	11,570,444	C/C	A/A
C1	11,281,475	C/C	A/A	C2	10,245,718	G/G	A/A	C3	4,790,890	C/C	T/T	C3	43,621,334	T/T	C/C	C4	42,054,007	G/G	T/T	C5	23,498,203	A/A	C/C	C6	27,904,457	G/G	A/A	C7	34,636,912	T/T	C/C	C8	27,796,504	G/G	T/T	C9	11,661,935	C/C	T/T
C1	11,336,678	A/A	G/G	C2	10,277,286	A/A	C/C	C3	4,799,264	A/A	G/G	C3	43,621,359	A/A	C/C	C4	42,054,380	C/C	T/T	C5	23,858,245	T/T	G/G	C6	27,904,473	A/A	C/C	C7	34,719,340	C/C	G/G	C8	27,796,570	G/G	T/T	C9	11,661,989	G/G	T/T
C1	11,336,679	C/C	T/T	C2	10,277,291	C/C	T/T	C3	4,799,304	A/A	G/G	C3	43,625,558	C/C	T/T	C4	42,054,435	A/A	G/G	C5	23,892,817	T/T	A/A	C6	27,904,534	G/G	A/A	C7	34,740,469	G/G	A/A	C8	27,909,705	G/G	A/A	C9	11,859,520	A/A	G/G
C1	11,400,542	C/C	T/T	C2	10,277,305	C/C	A/A	C3	4,820,860	T/T	C/C	C3	43,640,581	T/T	A/A	C4	42,081,382	A/A	C/C	C5	23,953,073	C/C	G/G	C6	27,904,556	T/T	A/A	C7	34,769,375	T/T	A/A	C8	27,918,129	G/G	A/A	C9	11,859,521	T/T	G/G
C1	11,450,277	T/T	C/C	C2	10,360,938	G/G	C/C	C3	4,820,872	T/T	A/A	C3	43,709,495	T/T	A/A	C4	42,148,910	A/A	G/G	C5	23,953,132	A/A	T/T	C6	27,904,615	G/G	A/A	C7	34,769,408	T/T	A/A	C8	27,918,145	A/A	G/G	C9	11,859,522	T/T	C/C
C1	11,461,520	A/A	T/T	C2	10,361,037	C/C	G/G	C3	4,965,040	C/C	T/T	C3	43,786,189	A/A	G/G	C4	42,275,990	T/T	C/C	C5	23,985,832	G/G	T/T	C6	27,943,912	G/G	C/C	C7	34,773,029	A/A	C/C	C8	28,054,106	G/G	A/A	C9	12,144,587	A/A	T/T
C1	11,461,616	G/G	A/A	C2	10,392,737	T/T	A/A	C3	4,996,537	G/G	A/A	C3	43,854,978	A/A	T/T	C4	42,290,346	G/G	A/A	C5	23,995,207	C/C	A/A	C6	28,055,683	T/T	A/A	C7	34,830,757	C/C	A/A	C8	28,054,110	G/G	A/A	C9	12,851,470	A/A	G/G
C1	11,461,633	C/C	A/A	C2	10,392,762	C/C	T/T	C3	5,048,222	A/A	C/C	C3	43,865,574	G/G	T/T	C4	42,290,401	C/C	T/T	C5	23,995,282	C/C	T/T	C6	28,055,694	T/T	C/C	C7	34,842,957	A/A	C/C	C8	28,054,122	A/A	C/C	C9	13,137,421	G/G	T/T
C1	11,461,656	T/T	C/C	C2	10,392,862	G/G	A/A	C3	5,048,228	A/A	G/G	C3	43,908,986	A/A	G/G	C4	42,644,290	C/C	A/A	C5	23,998,051	T/T	C/C	C6	28,140,658	G/G	C/C	C7	35,135,589	T/T	C/C	C8	28,054,133	A/A	G/G	C9	13,213,053	T/T	C/C
C1	11,461,728	T/T	A/A	C2	10,400,899	A/A	G/G	C3	5,048,369	C/C	T/T	C3	43,924,773	C/C	A/A	C4	42,628,727	G/G	C/C	C5	24,085,339	G/G	A/A	C6	28,157,458	C/C	T/T	C7	35,144,643	T/T	A/A	C8	28,279,145	A/A	G/G	C9	13,214,428	G/G	C/C
C1	11,505,628	A/A	T/T	C2	10,400,922	A/A	C/C	C3	5,066,086	C/C	T/T	C3	44,011,745	T/T	C/C	C4	42,828,734	G/G	T/T	C5	24,085,349	G/G	A/A	C6	28,185,695	T/T	A/A	C7	35,144,708	C/C	T/T	C8	28,379,148	C/C	G/G	C9	13,228,898	T/T	A/A
C1	11,517,336	A/A	T/T	C2	10,633,350	A/A	T/T	C3	5,066,088	A/A	G/G	C3	44,055,035	C/C	T/T	C4	42,844,105	A/A	G/G	C5	24,159,653	T/T	G/G	C6	28,196,921	T/T	C/C	C7	35,165,086	A/A	G/G	C8	28,379,172	T/T	C/C	C9	13,230,109	C/C	G/G
C1	11,517,339	A/A	T/T	C2	10,633,353	A/A	G/G	C3	5,066,107	T/T	C/C	C3	44,055,059	A/A	C/C	C4	42,867,337	C/C	T/T	C5	24,159,668	A/A	C/C	C6	28,196,937	A/A	C/C	C7	35,178,966	C/C	T/T	C8	28,423,230	A/A	C/C	C9	13,642,380	C/C	A/A
C1	11,533,649	C/C	T/T	C2	10,633,371	G/G	A/A	C3	5,067,999	A/A	G/G	C3	44,122,637	T/T	C/C	C4	42,946,221	T/T	C/C	C5	24,242,243	C/C	T/T	C6	28,196,960	A/A	T/T	C7	35,420,845	T/T	C/C	C8	28,423,269	G/G	T/T	C9	13,827,003	A/A	C/C
C1	11,651,037	T/T	G/G	C2	10,633,377	A/A	G/G	C3	5,070,201	T/T	C/C	C3	44,126,441	A/A	G/G	C4	42,946,324	C/C	T/T	C5	24,242,277	G/G	T/T	C6	28,197,018	G/G	C/C	C7	35,451,426	C/C	T/T	C8	28,516,945	A/A	G/G	C9	13,935,936	A/A	G/G
C1	11,651,121	A/A	G/G	C2	10,640,075	T/T	G/G	C3	5,073,749	A/A	C/C	C3	44,202,878	T/T	C/C	C4	42,956,988	G/G	C/C	C5	24,242,382	T/T	A/A	C6	28,197,064	A/A	C/C	C7	35,451,427	A/A	C/C	C8	28,620,210	G/G	T/T	C9	13,951,010	T/T	G/G
C1	11,705,934	G/G	A/A	C2	10,676,298	C/C	T/T	C3	5,073,751	G/G	T/T	C3	44,313,204	T/T	A/A	C4	43,011,499	C/C	G/G	C5	24,253,284	A/A	G/G	C6	28,369,468	C/C	G/G	C7	35,451,597	T/T	C/C	C8	28,797,207	T/T	A/A	C9	13,951,012	T/T	C/C
C1	11,724,311	C/C	A/A	C2	10,757,838	G/G	C/C	C3	5,073,806	C/C	T/T	C3	44,347,330	G/G	A/A	C4	43,011,534	A/A	G/G	C5	24,253,317	C/C	T/T	C6	28,537,467	T/T	G/G	C7	35,526,182	A/A	G/G	C8	28,810,295	A/A	G/G	C9	13,979,893	A/A	T/T
C1	11,724,526	A/A	G/G	C2	10,910,964	C/C	T/T	C3	5,099,294	C/C	A/A	C3	44,416,537	T/T	G/G	C4	43,011,609	C/C	T/T	C5	24,330,955	T/T	C/C	C6	28,544,162	A/A	C/C	C7	35,526,197	G/G	A/A	C8	29,014,062	G/G	A/A	C9	14,025,530	G/G	A/A
C1	11,739,710	T/T	C/C	C2	11,092,465	A/A	T/T	C3	5,099,332	T/T	A/A	C3	44,433,517	A/A	G/G	C4	43,011,642	C/C	T/T	C5	24,345,771	C/C	T/T	C6	28,544,163	G/G	A/A	C7	35,557,913	T/T	C/C	C8	29,014,089	T/T	C/C	C9	14,469,902	T/T	G/G
C1	11,739,777	A/A	G/G	C2	11,098,208	T/T	C/C	C3	5,099,356	A/A	G/G	C3	44,451,393	A/A	C/C	C4	43,051,948	C/C	T/T	C5	24,345,776	T/T	G/G	C6	28,544,274	C/C	T/T	C7	35,557,923	A/A	C/C	C8	29,014,128	G/G	A/A	C9	14,469,909	C/C	T/T
C1	11,806,156	T/T	A/A	C2	11,122,009	G/G	A/A	C3	5,099,369	G/G	C/C	C3	44,535,591	C/C	A/A	C4	43,051,971	T/T	A/A	C5	24,387,628	T/T	C/C	C6	28,544,280	C/C	T/T	C7	35,720,709	G/G	T/T	C8	29,014,134	T/T	G/G	C9	14,471,437	A/A	C/C
C1	11,865,165	A/A	G/G	C2	11,165,693	A/A	G/G	C3	5,206,895	G/G	A/A	C3	44,537,572	G/G	T/T	C4	43,072,924	A/A	C/C	C5	24,387,637	C/C	G/G	C6	28,544,306	C/C	G/G	C7	35,726,348	T/T	C/C	C8	29,014,143	A/A	G/G	C9	14,489,423	C/C	G/G
C1	11,865,176	A/A	G/G	C2	11,416,516	T/T	G/G	C3	5,206,918	C/C	T/T	C3	44,607,547	T/T	C/C	C4	43,073,120	T/T	C/C	C5	24,433,681	G/G	A/A	C6	28,598,191	C/C	T/T	C7	35,896,876	G/G	A/A	C8	29,023,600	C/C	T/T	C9	14,489,459	T/T	C/C
C1	11,865,206	G/G	A/A	C2	11,636,542	T/T	A/A	C3	5,228,470	A/A	G/G	C3	44,718,349	T/T	A/A	C4	43,073,156	G/G	A/A	C5	24,433,797	G/G	A/A	C6	28,723,457	C/C	T/T	C7	35,934,293	A/A	G/G								

Appendix III *Cont.*

Table S3: *Cont.* : Differences in Allelic variants at various chromosomes positions in parent lines; the cultivated rapid cycling DHSL150 and C07007.

	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.				
C1	12,734,905	G/G	A/A	C2	13,207,490	A/A	T/T	C3	5,733,235	T/T	A/A	C3	45,635,870	G/G	A/A	C4	44,803,850	C/C	T/T	C5	26,373,457	A/A	T/T	C6	31,169,801	T/T	C/A	C7	37,277,768	G/G	A/A	C8	30,463,256	A/A	C/C	C9	16,481,415	T/T	A/A	C/C
C1	12,734,950	G/G	A/A	C2	13,222,378	A/A	T/T	C3	5,749,115	T/T	A/A	C3	45,655,301	T/T	A/A	C4	45,319,494	T/T	C/C	C5	26,389,529	A/A	T/T	C6	31,211,172	G/G	A/A	C7	37,288,313	C/C	T/T	C8	30,489,247	A/A	C/C	C9	16,481,457	G/G	A/A	C/C
C1	12,734,956	C/C	T/T	C2	13,222,399	T/T	A/A	C3	5,748,116	T/T	C/C	C3	45,655,332	A/A	T/T	C4	45,389,683	C/C	G/G	C5	26,391,512	T/T	C/C	C6	31,215,005	C/C	T/T	C7	37,300,411	A/A	C/C	C8	30,601,654	G/G	C/C	C9	16,482,121	C/C	G/G	A/A
C1	12,770,133	G/G	T/T	C2	13,222,404	C/A	T/T	C3	5,882,854	A/A	G/G	C3	45,883,437	A/A	C/C	C4	45,389,714	G/G	A/A	C5	26,391,584	G/G	A/A	C6	31,247,221	A/A	G/G	C7	37,305,114	A/A	G/G	C8	30,601,676	T/T	C/C	C9	16,526,070	G/G	A/A	C/G
C1	12,770,186	G/G	T/T	C2	13,222,424	A/A	G/G	C3	5,943,522	T/T	C/C	C3	45,683,547	A/A	C/C	C4	45,725,296	A/A	G/G	C5	26,478,293	C/C	T/T	C6	31,247,233	T/T	A/A	C7	37,326,788	G/G	T/T	C8	30,601,715	T/T	C/C	C9	16,552,558	A/A	A/A	G/G
C1	12,770,204	C/C	T/T	C2	13,222,454	C/C	T/T	C3	6,039,464	C/C	T/T	C3	45,729,650	T/T	C/C	C4	45,728,116	C/C	A/A	C5	26,474,326	C/A	C/C	C6	31,384,551	A/A	T/T	C7	37,326,836	A/A	G/G	C8	30,602,411	T/T	C/C	C9	16,628,479	C/C	G/G	A/A
C1	12,770,205	C/C	T/T	C2	13,845,367	T/T	C/C	C3	6,048,374	T/T	C/C	C3	45,748,264	T/T	A/A	C4	45,979,815	C/C	T/T	C5	26,525,768	C/C	T/T	C6	31,696,032	T/T	A/A	C7	37,378,103	G/G	C/C	C8	30,603,891	G/G	C/C	C9	16,637,494	C/C	T/T	A/A
C1	12,770,222	T/T	G/G	C2	13,845,388	T/T	G/G	C3	6,119,736	A/A	G/G	C3	45,825,413	A/A	G/G	C4	45,979,879	C/C	A/A	C5	26,525,878	G/G	T/T	C6	31,696,048	T/T	C/C	C7	37,385,582	A/A	G/G	C8	30,604,043	T/T	G/G	C9	16,725,574	G/G	T/T	A/A
C1	12,770,243	A/A	T/T	C2	13,927,785	T/T	G/G	C3	6,132,319	A/A	G/G	C3	45,971,494	C/C	T/T	C4	45,979,890	G/G	T/T	C5	26,525,906	T/T	C/C	C6	31,696,082	C/C	G/G	C7	37,562,885	T/T	A/A	C8	30,604,142	T/T	A/A	C9	16,732,052	C/C	T/T	A/A
C1	12,770,270	A/A	G/G	C2	13,927,802	G/G	C/C	C3	6,132,324	C/C	G/G	C3	45,971,511	A/A	C/C	C4	45,979,924	T/T	A/A	C5	26,525,945	G/G	A/A	C6	32,150,423	T/T	C/C	C7	37,562,936	T/T	A/A	C8	30,643,171	A/A	C/C	C9	16,758,567	G/G	A/A	A/A
C1	12,771,217	G/G	C/C	C2	14,066,096	C/C	T/T	C3	6,156,026	C/C	G/G	C3	46,067,447	T/T	C/C	C4	45,979,932	T/T	C/C	C5	26,525,946	T/T	C/C	C6	32,150,560	T/T	A/A	C7	37,596,872	A/A	C/C	C8	30,643,174	T/T	A/A	C9	16,762,840	A/A	G/G	A/A
C1	12,771,422	G/A	C/A	C2	14,079,924	A/A	T/T	C3	6,156,029	T/T	A/A	C3	46,118,734	A/A	G/G	C4	45,983,272	T/T	G/G	C5	26,549,517	C/C	T/T	C6	32,150,588	C/C	T/T	C7	37,596,887	C/C	T/T	C8	30,646,491	G/G	T/T	C9	16,762,920	G/G	C/C	A/A
C1	12,778,128	T/T	G/G	C2	14,158,301	T/T	C/C	C3	6,220,560	C/C	A/A	C3	46,138,184	C/C	A/A	C4	45,983,295	C/C	T/T	C5	26,779,879	A/A	G/G	C6	32,164,273	G/G	C/C	C7	37,596,944	A/A	C/C	C8	30,646,514	T/T	C/C	C9	16,869,101	C/C	T/T	A/A
C1	13,199,262	A/A	T/T	C2	14,158,344	G/G	A/A	C3	6,274,086	C/C	T/T	C3	46,214,258	C/C	T/T	C4	46,057,986	C/C	T/T	C5	26,820,997	A/A	C/C	C6	32,316,939	C/C	T/T	C7	37,596,949	A/A	C/C	C8	30,685,417	G/G	A/A	C9	16,869,743	T/T	C/C	A/A
C1	13,219,326	C/A	T/T	C2	14,184,636	C/C	T/T	C3	6,274,098	C/C	T/T	C3	46,838,145	C/C	T/T	C4	46,096,771	C/C	T/T	C5	26,901,430	T/T	G/G	C6	32,422,830	A/A	C/C	C7	37,601,428	T/T	C/C	C8	30,690,376	T/T	C/C	C9	16,869,973	C/C	T/T	A/A
C1	13,281,494	T/T	C/A	C2	14,247,744	G/G	A/A	C3	6,295,940	A/A	G/G	C3	46,867,065	A/A	C/C	C4	46,097,093	C/C	T/T	C5	27,007,057	G/G	A/A	C6	32,645,614	T/T	A/A	C7	37,622,315	A/A	C/C	C8	30,690,381	T/T	A/A	C9	16,879,041	C/C	T/T	A/A
C1	13,306,691	G/G	A/A	C2	14,503,835	T/T	C/C	C3	6,295,979	T/T	G/G	C3	46,867,124	G/G	C/C	C4	46,136,636	A/A	G/G	C5	27,333,522	T/T	A/A	C6	32,778,675	G/G	A/A	C7	37,622,318	G/G	A/A	C8	30,728,250	T/T	G/G	C9	16,905,281	G/G	A/A	A/A
C1	13,339,446	A/A	G/G	C2	14,564,135	T/T	C/C	C3	6,295,994	T/T	C/C	C3	46,936,838	G/G	T/T	C4	46,136,643	T/T	C/C	C5	27,333,534	C/C	T/T	C6	32,810,626	T/T	G/G	C7	37,622,366	G/G	A/A	C8	31,032,346	G/G	A/A	C9	16,905,299	G/G	C/C	A/A
C1	13,339,494	A/A	T/T	C2	14,591,055	G/G	A/A	C3	6,302,288	G/G	A/A	C3	46,936,922	T/T	A/A	C4	46,137,192	C/C	T/T	C5	27,467,080	A/A	C/C	C6	32,810,752	T/T	C/C	C7	37,633,536	A/A	C/C	C8	31,041,194	C/A	A/A	C9	16,996,128	C/C	T/T	A/A
C1	13,339,569	G/G	T/T	C2	14,591,095	T/T	C/C	C3	6,317,642	C/C	T/T	C3	47,032,543	T/T	C/C	C4	46,137,227	T/T	C/C	C5	27,467,093	C/C	T/T	C6	32,810,910	A/A	C/C	C7	37,633,552	C/C	T/T	C8	31,095,711	A/A	G/G	C9	17,014,079	A/A	A/A	G/G
C1	13,392,176	C/C	T/T	C2	14,600,447	G/G	T/T	C3	6,319,530	G/G	A/A	C3	48,876,509	T/T	A/A	C4	46,137,249	T/T	C/C	C5	27,563,979	G/G	T/T	C6	33,017,770	T/T	C/C	C7	37,633,556	T/T	C/C	C8	31,095,717	T/T	C/C	C9	17,014,113	A/A	A/A	G/G
C1	13,434,527	C/C	T/T	C2	14,603,036	C/C	A/A	C3	6,319,557	A/A	G/G	C3	48,913,860	C/C	A/A	C4	46,137,270	C/C	G/G	C5	27,563,987	T/T	C/C	C6	33,017,782	C/C	T/T	C7	37,635,482	G/G	A/A	C8	31,351,701	C/C	A/A	C9	17,166,109	T/T	G/G	A/A
C1	13,434,531	A/A	G/G	C2	14,676,044	G/G	A/A	C3	6,319,565	T/T	A/A	C3	48,913,875	A/A	C/C	C4	46,248,851	A/A	C/C	C5	27,570,459	C/C	A/A	C6	33,037,617	A/A	G/G	C7	37,636,151	G/G	A/A	C8	31,471,869	C/C	T/T	C9	17,585,388	G/G	A/A	T/T
C1	14,036,225	T/T	C/C	C2	14,676,056	A/A	C/C	C3	6,320,005	C/C	T/T	C3	48,955,483	C/C	A/A	C4	46,248,886	G/G	A/A	C5	27,602,465	T/T	C/C	C6	33,456,761	T/T	G/G	C7	37,636,169	T/T	C/C	C8	31,557,217	C/C	A/A	C9	17,566,104	C/C	A/A	A/A
C1	14,036,237	G/G	T/T	C2	14,676,089	G/G	C/C	C3	6,320,011	A/A	G/G	C3	48,955,493	C/C	T/T	C4	46,267,114	T/T	A/A	C5	27,602,473	T/T	G/G	C6	33,628,615	A/A	C/C	C7	37,668,039	G/G	A/A	C8	31,613,406	T/T	C/C	C9	18,065,765	A/A	A/A	G/G
C1	14,037,089	G/G	T/T	C2	14,898,724	G/G	A/A	C3	6,320,038	C/C	T/T	C3	48,999,085	C/C	T/T	C4	46,288,334	C/C	T/T	C5	27,758,178	T/T	A/A	C6	33,918,383	A/A	G/G	C7	37,758,126	T/T	C/C	C8	31,613,427	A/A	G/G	C9	18,344,444	T/T	G/G	A/A
C1	14,074,692	C/C	G/G	C2	14,904,936	G/G	C/C	C3	6,321,289	G/G	A/A	C3	48,999,129	A/A	G/G	C4	46,288,347	C/C	T/T	C5	27,758,241	T/T	A/A	C6	34,017,260	C/C	A/A	C7	37,873,312	T/T	A/A	C8	31,613,442	G/G	T/T	C9	19,359,292	G/G	A/A	A/A
C1	14,074,741	C/C	T/T	C2	14,949,109	C/C	T/T	C3	6,340,116	G/G	A/A	C3	49,083,494	A/A	T/T	C4	46,288,370	A/A	G/G	C5	27,779,662	T/T	A/A	C6	34,084,437	A/A	G/G	C7	37,875,232	A/A	G/G	C8	31,699,365	C/C	T/T	C9	20,410,573	A/A	C/C	A/A
C1	14,120,307	C/C	T/T	C2	15,108,804	T/T	C/C	C3	6,369,291	A/A	T/T	C3	49,083,561	C/C	T/T	C4	46,288,396	G/G	T/T	C5	27,787,982	G/G	A/A	C6	34,094,513	T/T	C/C	C7	37,907,516	A/A	C/C	C8	31,704,195	C/C	T/T	C9	20,410,576	G/G	T/T	A/A
C1	14,230,363	T/T	A/A	C2	15,108,848	G/G	T/T	C3	6,369,391	T/T	A/A	C3	49,083,592	T/T	A/A	C4	46,288,408	A/A	G/G	C5	27,788,000	G/G	A/A	C6	34,094,603	A/A	G/G	C7	37,920,957	A/A	G/G	C8	31,704,218	T/T	C/C	C9	20,410,600	T/T	G/G	A/A
C1	14,304,066	C/C	T/T	C2	15,108,903	T/T	G/G	C3	6,369,417	G/G	A/A	C3	49,447,610	C/C	A/A	C4	46,288,409	T/T	A/A	C5	27,791,243	G/G	A/A	C6	34,094,620	G/G	A/A	C7	37,999,238	G/G	T/T	C8	31,704,304	T/T	G/G	C9	20,439,511	C/C	A/A	A/A
C1	14,313,650	C/C	T/T	C2	15,251,840	A/A	T/T	C3	6,369,429	T/T	A/A	C3	49,453,336	T/T	A/A	C4	46,610,404	C/C	T/T	C5	27,791,261	T/T	C/C	C6	34,097,416	G/G	T/T	C7	38,030,209	G/G	A/A	C8	31,704,318	A/A	G/G	C9	20,542,127	G/G	A/A	A/A
C1	14,313,653	G/G	T/T	C2	15,251,841	T/T	A/A	C3	6,383,243	T/T	C/C	C3	49,729,609	A/A	C/C	C4	46,673,560	C/C	G/G	C5	27,791,274	C/C	T/T	C6	34,097,550	T/T	C/C	C7	38,030,211	A/A	C/C	C8	31,848,773	A/A	C/C	C9	20,592,289	A/A	G/G	A/A
C1	14,313,767	T/T	G/G	C2	15,252,599	G/G	A/A	C3	6,383,317	A/A	G/G	C3	49,855,554	T/T	C/C	C4	46,673,562	T/T	C/C	C5	27,785,439	T/T	C/C	C6	34,468,931	C/C	A/A	C7	38,031,692	G/G	A/A	C8	31,874,202	T/T	C/C	C9	20,669,740	T/T	G/G	A/A
C1	14,389,131	A/A	G/G	C2	15,252,610	A/A	G/G	C3	6,383,383	C/C	T/T	C3	49,855,652	G/G	A/A	C4	46,697,178	G/G	A/A	C5	27,786,793	T/T	C/C	C6	34,480,983	A/A	C/C	C7	38,038,358	T/T	A/A	C8	31,874,230	T/T	G/G	C9	20,669,741	C/C	A/A	A/A
C1	14,449,414	C/C	T/T	C2	15,327,513	A/A	G/G	C3	6,383,413	C/C	T/T	C3	49,923,819	T/T	C/C	C4	46,697,332	C/C	T/T	C5	27,821,722	T/T	C/C	C6	34,502,347															

* Note: Yellow colour indicates variants sequence of the cultivated rapid cycling DHSL150 line and grey colour indicates that of wild S1 C07007 line showing differences in allelic variant in their chromosomes.

Appendix III *Cont.***Table S3: *Cont.* : Differences in Allelic variants at various chromosomes positions in parent lines; the cultivated rapid cycling DHSL150 and C07007.**

Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007												
C1	15,046,802	T/T	A/A	C2	16,851,473	A/A	C/C	C3	6,825,544	T/T	G/G	C3	51,012,688	G/G	T/T	C4	47,484,195	G/G	A/A	C5	28,165,395	T/T	G/G	C6	35,898,638	C/C	T/T	C7	38,611,754	A/A	C/C	C8	32,349,647	G/G	C/C	C9	22,759,836	C/C	T/T
C1	15,048,574	A/A	C/C	C2	16,853,120	A/A	C/C	C3	6,834,299	T/T	C/C	C3	51,012,706	A/A	G/G	C4	47,484,268	T/T	C/C	C5	28,210,962	G/G	C/C	C6	35,898,673	A/A	C/C	C7	38,627,419	T/T	G/G	C8	32,377,604	C/C	G/G	C9	22,759,857	C/C	T/T
C1	15,049,952	C/C	G/G	C2	16,994,975	C/C	T/T	C3	6,834,387	T/T	C/C	C3	51,013,216	C/C	G/G	C4	47,537,918	G/G	A/A	C5	28,210,990	C/C	A/A	C6	35,898,674	C/C	G/G	C7	38,691,315	T/T	C/C	C8	32,413,033	C/C	T/T	C9	22,759,869	G/G	A/A
C1	15,052,615	T/T	C/C	C2	16,994,993	T/T	C/C	C3	7,196,672	A/A	T/T	C3	51,099,017	T/T	G/G	C4	47,769,134	A/A	C/C	C5	28,212,834	G/G	A/A	C6	35,922,561	G/G	A/A	C7	38,697,503	A/A	G/G	C8	32,425,571	T/T	C/C	C9	22,765,566	T/T	A/A
C1	15,052,628	G/G	C/C	C2	16,998,613	A/A	G/G	C3	7,246,844	A/A	C/C	C3	51,199,219	T/T	C/C	C4	47,776,344	A/A	T/T	C5	28,215,654	G/G	A/A	C6	35,922,711	A/A	G/G	C7	38,826,119	C/C	T/T	C8	32,435,737	G/G	A/A	C9	22,782,391	T/T	C/C
C1	15,076,563	C/C	A/A	C2	16,998,625	G/G	C/C	C3	7,275,852	A/A	T/T	C3	51,199,229	G/G	A/A	C4	47,899,315	C/C	T/T	C5	28,864,596	T/T	C/C	C6	35,923,091	G/G	A/A	C7	38,826,140	G/G	C/C	C8	32,441,503	G/G	T/T	C9	22,922,905	T/T	C/C
C1	15,076,596	A/A	T/T	C2	16,998,636	C/C	A/A	C3	7,368,241	A/A	G/G	C3	51,199,261	C/C	A/A	C4	47,911,735	T/T	C/C	C5	28,926,939	C/C	T/T	C6	35,923,100	G/G	A/A	C7	38,854,502	G/G	T/T	C8	32,444,375	C/C	A/A	C9	22,997,122	A/A	T/T
C1	15,738,826	A/A	T/T	C2	17,044,040	G/G	T/T	C3	7,368,260	C/C	T/T	C3	51,317,382	C/C	T/T	C4	47,922,833	C/C	A/A	C5	28,949,780	A/A	G/G	C6	35,923,121	G/G	A/A	C7	38,888,875	G/G	A/A	C8	32,455,031	T/T	A/A	C9	22,997,125	T/T	C/C
C1	15,738,868	C/C	A/A	C2	17,044,043	A/A	T/T	C3	7,368,391	T/T	G/G	C3	51,317,413	A/A	G/G	C4	47,938,853	A/A	C/C	C5	28,949,884	G/G	A/A	C6	35,923,155	C/C	G/G	C7	39,094,162	C/C	T/T	C8	32,455,032	C/C	A/A	C9	22,997,371	A/A	G/G
C1	15,739,054	C/C	T/T	C2	17,076,430	A/A	C/C	C3	7,593,819	G/G	A/A	C3	51,317,419	T/T	A/A	C4	47,938,853	A/A	G/G	C5	28,949,894	A/A	G/G	C6	35,923,165	C/C	T/T	C7	39,169,530	C/C	T/T	C8	32,490,671	T/T	C/C	C9	23,066,981	C/C	A/A
C1	15,751,338	T/T	A/A	C2	17,779,534	G/G	A/A	C3	7,593,860	C/C	T/T	C3	51,333,371	C/C	T/T	C4	47,948,487	T/T	G/G	C5	28,949,915	T/T	C/C	C6	35,923,236	C/C	A/A	C7	39,169,539	G/G	C/C	C8	32,514,200	T/T	C/C	C9	23,067,181	A/A	G/G
C1	15,751,440	A/A	G/G	C2	17,779,568	T/T	C/C	C3	7,607,088	C/C	A/A	C3	51,333,388	T/T	C/C	C4	47,948,631	G/G	T/T	C5	28,953,191	T/T	C/C	C6	35,923,277	A/A	C/C	C7	39,305,624	A/A	G/G	C8	32,528,373	G/G	A/A	C9	23,067,335	G/G	A/A
C1	15,789,128	A/A	G/G	C2	17,791,319	A/A	G/G	C3	7,607,137	C/C	A/A	C3	51,475,937	G/G	C/C	C4	48,001,917	G/G	A/A	C5	28,953,198	G/G	T/T	C6	35,949,374	G/G	C/C	C7	39,340,455	G/G	A/A	C8	32,587,215	A/A	G/G	C9	23,069,591	A/A	C/C
C1	15,965,793	G/G	A/A	C2	17,791,973	A/A	T/T	C3	7,616,668	C/C	T/T	C3	51,496,349	A/A	G/G	C4	48,081,542	A/A	T/T	C5	28,953,236	C/C	T/T	C6	36,104,428	T/T	C/C	C7	39,396,676	A/A	C/C	C8	32,593,241	T/T	C/C	C9	23,069,601	C/C	A/A
C1	15,965,841	C/C	T/T	C2	17,791,976	T/T	C/C	C3	7,616,682	A/A	G/G	C3	51,800,047	C/C	T/T	C4	48,081,581	C/C	A/A	C5	28,953,250	A/A	G/G	C6	36,104,476	T/T	A/A	C7	39,530,721	A/A	T/T	C8	32,668,611	C/C	T/T	C9	23,337,553	G/G	A/A
C1	15,965,875	A/A	G/G	C2	17,792,065	A/A	T/T	C3	7,616,786	A/A	C/C	C3	51,800,400	A/A	G/G	C4	48,098,606	A/A	T/T	C5	28,953,259	A/A	G/G	C6	36,111,397	G/G	C/C	C7	39,688,300	A/A	G/G	C8	32,881,951	T/T	C/C	C9	23,369,955	T/T	C/C
C1	15,977,222	G/G	C/C	C2	17,993,438	G/G	C/C	C3	7,959,361	G/G	A/A	C3	51,800,412	A/A	G/G	C4	48,150,617	T/T	G/G	C5	29,003,658	T/T	A/A	C6	36,117,963	G/G	A/A	C7	39,688,302	A/A	G/G	C8	32,949,890	A/A	C/C	C9	23,373,270	C/C	T/T
C1	16,084,347	T/T	C/C	C2	17,993,488	A/A	T/T	C3	8,151,024	T/T	C/C	C3	51,802,295	C/C	A/A	C4	48,158,238	T/T	A/A	C5	29,071,420	G/G	A/A	C6	36,117,988	C/C	A/A	C7	39,720,978	T/T	C/C	C8	32,949,935	T/T	A/A	C9	23,374,221	C/C	A/A
C1	16,304,527	C/C	T/T	C2	17,993,496	G/G	T/T	C3	8,151,066	A/A	G/G	C3	51,832,730	G/G	C/C	C4	48,199,496	A/A	G/G	C5	29,071,469	A/A	G/G	C6	36,148,763	A/A	G/G	C7	39,721,011	T/T	C/C	C8	32,949,976	A/A	G/G	C9	23,413,917	T/T	C/C
C1	16,304,586	A/A	G/G	C2	18,236,970	T/T	G/G	C3	8,206,956	G/G	A/A	C3	51,832,748	A/A	C/C	C4	48,200,823	A/A	G/G	C5	29,071,568	C/C	T/T	C6	36,271,043	A/A	G/G	C7	39,722,298	T/T	C/C	C8	32,961,342	T/T	C/C	C9	23,426,142	A/A	C/C
C1	16,377,063	A/A	C/C	C2	18,236,993	T/T	C/C	C3	8,393,981	C/C	T/T	C3	51,952,582	C/C	G/G	C4	48,259,359	C/C	T/T	C5	29,071,584	C/C	A/A	C6	36,271,048	A/A	G/G	C7	39,782,699	G/G	A/A	C8	33,043,889	A/A	C/C	C9	23,426,160	C/C	T/T
C1	16,400,552	A/A	G/G	C2	18,242,694	A/A	G/G	C3	8,507,382	T/T	C/C	C3	52,446,053	A/A	T/T	C4	48,295,366	T/T	C/C	C5	29,380,262	C/C	T/T	C6	36,271,205	C/C	T/T	C7	39,789,788	C/C	G/A	C8	33,043,910	C/C	T/T	C9	23,444,655	T/T	G/G
C1	16,452,089	T/T	A/A	C2	18,242,713	T/T	G/G	C3	8,507,405	T/T	C/C	C3	52,701,005	C/C	T/T	C4	48,417,631	C/C	T/T	C5	29,419,802	T/T	C/C	C6	36,271,208	A/A	C/C	C7	39,789,800	G/G	A/A	C8	33,093,076	G/G	T/T	C9	23,444,667	A/A	G/G
C1	16,569,679	A/A	G/G	C2	18,242,829	T/T	C/C	C3	8,519,194	C/C	G/G	C3	52,868,679	C/C	T/T	C4	48,458,669	G/G	T/T	C5	29,531,698	T/T	C/C	C6	36,317,668	G/G	C/C	C7	39,789,801	A/A	T/T	C8	33,093,154	T/T	A/A	C9	23,481,916	T/T	C/C
C1	16,692,402	T/T	G/G	C2	18,300,245	C/C	T/T	C3	8,763,142	G/G	A/A	C3	52,973,364	A/A	G/G	C4	48,687,192	A/A	C/C	C5	29,963,729	C/C	G/G	C6	36,341,716	C/C	G/G	C7	40,042,042	A/A	G/G	C8	33,107,515	C/C	T/T	C9	23,504,820	T/T	A/A
C1	16,692,426	T/T	G/G	C2	18,308,509	G/G	C/C	C3	8,763,145	C/C	A/A	C3	52,977,476	G/G	C/C	C4	49,048,958	A/A	T/T	C5	30,019,304	G/G	A/A	C6	36,341,760	A/A	T/T	C7	40,070,436	C/C	T/T	C8	33,295,461	C/C	T/T	C9	23,541,323	C/C	A/A
C1	16,953,996	A/A	G/G	C2	18,308,544	T/T	C/C	C3	8,763,208	G/G	A/A	C3	53,144,378	A/A	G/G	C4	49,061,073	A/A	G/G	C5	30,123,593	A/A	T/T	C6	36,341,761	G/G	T/T	C7	40,162,744	G/G	T/T	C8	33,295,514	G/G	A/A	C9	23,578,146	C/C	A/A
C1	16,954,026	A/A	G/G	C2	18,506,749	A/A	G/G	C3	8,763,348	C/C	G/G	C3	53,144,450	C/C	A/A	C4	49,061,092	C/C	A/A	C5	30,297,272	A/A	T/T	C6	36,341,808	G/G	T/T	C7	40,250,814	G/G	A/A	C8	33,342,595	G/G	C/C	C9	23,578,234	A/A	G/G
C1	16,954,032	T/T	G/G	C2	18,506,750	T/T	A/A	C3	8,876,369	C/C	T/T	C3	53,167,980	T/T	C/C	C4	49,061,103	T/T	C/C	C5	30,473,559	A/A	G/G	C6	36,341,812	C/C	T/T	C7	40,317,879	G/G	A/A	C8	33,342,808	G/G	A/A	C9	23,583,036	T/T	C/C
C1	17,072,346	C/C	T/T	C2	18,508,867	A/A	T/T	C3	8,876,372	C/C	G/G	C3	53,168,027	T/T	G/G	C4	49,191,927	C/C	G/G	C5	30,547,779	A/A	C/C	C6	36,363,515	G/G	C/C	C7	40,393,232	C/C	T/T	C8	33,342,905	A/A	T/T	C9	23,667,381	T/T	C/C
C1	17,072,349	C/C	T/T	C2	18,520,916	A/A	T/T	C3	8,883,916	G/G	A/A	C3	53,248,252	A/A	G/G	C4	49,191,975	A/A	G/G	C5	30,746,853	A/A	T/T	C6	36,367,455	A/A	G/G	C7	40,458,663	A/A	T/T	C8	33,348,466	C/C	T/T	C9	23,730,228	C/C	A/A
C1	17,072,372	A/A	C/C	C2	18,555,285	C/C	T/T	C3	9,059,582	T/T	C/C	C3	53,428,095	T/T	G/G	C4	49,191,984	T/T	G/G	C5	30,932,277	G/G	T/T	C6	36,367,522	C/C	G/G	C7	40,461,702	A/A	C/C	C8	33,432,076	G/G	A/A	C9	23,751,850	G/G	A/A
C1	17,184,604	T/T	C/C	C2	18,555,360	T/T	A/A	C3	9,061,325	G/G	A/A	C3	53,466,645	T/T	A/A	C4	49,424,307	G/G	A/A	C5	30,932,354	A/A	T/T	C6	36,367,544	T/T	C/C	C7	40,653,115	T/T	C/C	C8	33,434,246	C/C	A/A	C9	23,787,289	A/A	G/G
C1	17,184,694	C/C	T/T	C2	18,629,873	G/G	T/T	C3	9,062,551	C/C	T/T	C3	53,485,976	G/G	T/T	C4	49,689,691	T/T	A/A	C5	30,946,185	G/G	A/A	C6	36,367,591	C/C	T/T	C7	40,759,602	G/G	C/C	C8	33,500,993	A/A	C/C	C9	23,797,312	G/G	A/A
C1	17,184,773	A/A	C/C	C2	18,671,064	G/G	T/T	C3	9,062,572	G/G	A/A	C3	53,521,130	T/T	C/C	C4	49,692,865	T/T	G/G	C5	30,946,206	C/C	A/A	C6	36,367,614	C/C	A/A	C7	40,910,966	G/G	A/A	C8	33,719,780	G/G	C/C	C9	23,955,441	A/A	G/G
C1	17,185,316	A/A	G/G	C2	18,727,145	T/T	C/C	C3	9,117,508	C/C	T/T	C3	53,521,149	C/C	T/T	C4	49,710,179	G/G	C/C	C5	30,946,238	T/T	C/C	C6	36,402,650	G/G	A/A	C7	40,912,648	T/T	G/G	C8	33,720,492	G					

Appendix III *Cont.***Table S3: *Cont.* : Differences in Allelic variants at various chromosomes positions in parent lines; the cultivated rapid cycling DHSL150 and C07007.**

Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007								
C1	18,184,466	C/C	T/T	C2	20,361,092	T/T	C/C	C3	9,899,649	C/C	G/G	C3	54,403,990	T/T	C/C	C4	49,856,523	A/A	T/T	C5	32,537,168	A/A	C/C	C6	36,454,643	T/T	C/C	C7	41,352,486	T/T	A/A	C8	33,833,261	A/A	G/G	C9	28,594,847	A/A	C/C
C1	18,234,998	C/C	T/T	C2	20,376,315	T/T	G/G	C3	9,944,110	C/C	T/T	C3	54,404,064	G/G	C/C	C4	49,863,583	A/A	G/G	C5	32,537,262	T/T	A/A	C6	36,454,702	G/G	A/A	C7	41,356,951	C/C	G/G	C8	33,833,361	A/A	G/G	C9	28,662,383	A/A	G/G
C1	18,259,531	A/A	C/C	C2	20,376,386	G/G	A/A	C3	9,944,122	T/T	A/A	C3	54,595,673	A/A	G/G	C4	49,876,182	C/C	T/T	C5	32,591,284	C/C	T/T	C6	36,454,705	C/C	T/T	C7	41,489,005	C/C	T/T	C8	33,837,275	G/G	A/A	C9	29,039,309	A/A	G/G
C1	18,341,425	T/T	A/A	C2	20,564,060	C/C	T/T	C3	9,944,143	A/A	C/C	C3	54,619,326	T/T	C/C	C4	49,878,516	C/C	A/A	C5	32,612,371	T/T	A/A	C6	36,454,757	C/C	G/G	C7	41,489,078	A/A	G/G	C8	33,837,338	G/G	C/C	C9	29,295,231	G/G	A/A
C1	18,360,393	A/A	C/C	C2	20,564,089	A/A	G/G	C3	9,980,664	C/C	T/T	C3	54,647,534	G/G	C/C	C4	50,003,194	A/A	G/G	C5	33,051,546	A/A	C/C	C6	36,730,766	T/T	A/A	C7	41,489,122	C/C	A/A	C8	33,869,918	G/G	A/A	C9	29,600,784	C/C	T/T
C1	18,488,347	G/G	C/C	C2	21,550,333	G/G	A/A	C3	10,014,200	G/G	A/A	C3	55,027,956	C/C	T/T	C4	50,175,434	C/C	T/T	C5	33,053,900	C/C	G/G	C6	36,734,367	A/A	T/T	C7	41,489,128	G/G	T/T	C8	33,870,586	C/C	T/T	C9	29,997,503	A/A	C/C
C1	18,495,143	G/G	A/A	C2	21,551,556	T/T	C/C	C3	10,014,296	A/A	G/G	C3	55,027,996	G/G	A/A	C4	50,348,003	G/G	A/A	C5	33,116,130	A/A	G/G	C6	36,734,479	G/G	A/A	C7	41,551,570	A/A	G/G	C8	33,870,966	T/T	C/C	C9	30,144,348	A/A	G/G
C1	18,495,159	C/C	A/A	C2	22,171,027	C/C	T/T	C3	10,180,594	G/G	A/A	C3	55,028,011	A/A	G/G	C4	50,348,021	C/C	T/T	C5	33,121,732	T/T	A/A	C6	36,734,511	A/A	G/G	C7	41,551,594	A/A	G/G	C8	33,880,522	T/T	C/C	C9	30,561,943	T/T	C/C
C1	18,495,190	G/G	A/A	C2	22,174,901	G/G	C/C	C3	10,195,933	G/G	A/A	C3	55,270,126	A/A	G/G	C4	50,540,636	A/A	G/G	C5	33,477,955	C/C	G/G	C6	36,750,902	G/G	A/A	C7	41,551,671	G/G	A/A	C8	33,880,537	C/C	G/G	C9	30,788,883	C/C	A/A
C1	18,526,413	C/C	A/A	C2	22,175,065	G/G	C/C	C3	10,228,057	C/C	G/G	C3	55,615,442	A/A	G/G	C4	50,679,260	C/C	A/A	C5	33,649,199	G/G	T/T	C6	36,766,431	G/G	C/C	C7	41,637,182	G/G	A/A	C8	33,880,645	G/G	T/T	C9	30,870,370	T/T	C/C
C1	18,527,151	A/A	G/G	C2	22,175,097	T/T	C/C	C3	10,402,582	T/T	C/C	C3	55,789,071	C/C	T/T	C4	50,679,299	G/G	C/C	C5	33,651,417	A/A	G/G	C6	36,823,080	T/T	A/A	C7	41,637,795	G/G	C/C	C8	33,896,235	T/T	A/A	C9	32,949,220	A/A	C/C
C1	18,564,344	G/G	T/T	C2	22,189,304	A/A	G/G	C3	10,402,624	C/C	G/G	C3	55,813,880	T/T	C/C	C4	50,682,846	A/A	T/T	C5	33,655,017	A/A	C/C	C6	36,849,406	G/G	A/A	C7	41,637,798	C/C	T/T	C8	33,927,994	A/A	T/T	C9	33,208,551	A/A	C/C
C1	18,610,292	C/C	T/T	C2	22,189,317	C/A	G/G	C3	10,418,778	C/C	A/A	C3	55,880,272	C/C	T/T	C4	50,782,266	C/C	A/A	C5	33,664,637	T/T	G/G	C6	36,849,598	T/T	A/A	C7	41,681,359	C/C	T/T	C8	33,928,195	G/G	A/A	C9	33,253,850	C/C	A/A
C1	18,693,684	G/G	A/A	C2	22,189,374	G/G	A/A	C3	10,418,779	T/T	C/C	C3	55,927,487	A/A	G/G	C4	50,797,237	T/T	G/G	C5	33,665,066	T/T	G/G	C6	36,849,606	C/C	G/G	C7	41,681,508	A/A	T/T	C8	33,928,676	C/C	G/G	C9	33,360,128	G/G	T/T
C1	18,699,942	T/T	C/C	C2	22,247,142	C/C	T/T	C3	10,422,479	A/A	C/C	C3	56,079,702	C/C	G/G	C4	50,797,239	T/T	A/A	C5	33,704,758	A/A	T/T	C6	36,972,592	T/T	A/A	C7	41,844,064	T/T	A/A	C8	33,928,711	G/G	A/A	C9	33,360,231	C/C	T/T
C1	18,699,954	G/G	T/T	C2	22,247,226	C/C	T/T	C3	10,449,659	A/A	T/T	C3	56,079,706	C/C	T/T	C4	50,797,258	C/C	T/T	C5	33,704,791	G/G	A/A	C6	36,974,798	T/T	C/C	C7	41,844,087	T/T	C/C	C8	33,928,817	G/G	A/A	C9	33,648,825	C/C	T/T
C1	23,090,797	C/C	T/T	C2	22,317,535	T/T	G/G	C3	10,449,678	A/A	C/C	C3	56,456,237	G/G	A/A	C4	51,180,689	C/C	A/A	C5	33,737,600	A/A	T/T	C6	36,974,846	C/C	T/T	C7	41,983,988	T/T	C/C	C8	33,928,864	C/C	T/T	C9	34,184,757	T/T	C/C
C1	23,145,776	A/A	G/G	C2	22,320,236	T/T	C/C	C3	10,464,195	C/C	A/A	C3	56,456,261	A/A	G/G	C4	51,195,493	C/C	G/G	C5	33,737,646	T/T	C/C	C6	36,983,143	T/T	C/C	C7	42,043,473	T/T	G/G	C8	33,928,888	C/C	T/T	C9	34,218,079	T/T	C/C
C1	23,145,821	G/G	A/A	C2	22,398,375	A/A	G/G	C3	10,489,097	C/C	T/T	C3	56,456,303	C/C	T/T	C4	51,195,600	A/A	C/C	C5	33,978,616	C/C	T/T	C6	36,983,185	G/G	A/A	C7	42,043,509	G/G	T/T	C8	33,942,955	G/G	A/A	C9	35,268,785	T/T	A/A
C1	23,337,272	C/C	T/T	C2	22,403,587	A/A	T/T	C3	10,530,215	G/G	C/C	C3	56,481,284	T/T	C/C	C4	51,279,064	C/C	T/T	C5	33,978,626	G/G	A/A	C6	36,983,263	G/G	A/A	C7	42,054,911	C/C	A/A	C8	33,968,319	A/A	G/G	C9	35,268,868	C/C	T/T
C1	23,420,387	A/A	T/T	C2	22,403,590	T/T	C/C	C3	10,530,291	G/G	A/A	C3	56,584,611	C/C	G/G	C4	51,365,287	T/T	C/C	C5	34,059,266	T/T	G/G	C6	36,984,379	A/A	G/G	C7	42,057,564	G/G	A/A	C8	33,969,103	T/T	A/A	C9	35,268,878	A/A	G/G
C1	23,488,393	T/T	C/C	C2	22,403,638	A/A	G/G	C3	10,751,409	G/G	T/T	C3	56,616,124	C/C	A/A	C4	51,365,300	T/T	G/G	C5	34,414,944	T/T	A/A	C6	36,984,394	T/T	G/G	C7	42,069,314	C/C	G/G	C8	33,982,201	C/C	T/T	C9	35,269,168	T/T	G/G
C1	23,614,237	A/A	G/G	C2	22,605,120	G/G	A/A	C3	10,790,436	A/A	C/C	C3	57,161,906	C/C	T/T	C4	51,450,019	C/C	G/G	C5	34,414,949	G/G	A/A	C6	36,991,538	G/G	A/A	C7	42,322,943	C/C	G/G	C8	33,982,234	G/G	A/A	C9	35,269,878	G/G	C/C
C1	23,926,406	G/G	C/C	C2	22,635,436	A/A	G/G	C3	10,847,693	T/T	C/C	C3	57,241,520	C/C	T/T	C4	51,454,804	A/A	T/T	C5	34,414,980	A/A	T/T	C6	36,991,544	G/G	A/A	C7	42,330,134	G/G	T/T	C8	33,982,297	C/C	T/T	C9	35,269,879	T/T	A/A
C1	24,133,123	T/T	G/G	C2	22,791,068	T/T	C/C	C3	10,847,694	C/C	A/A	C3	57,266,646	A/A	C/C	C4	51,454,811	A/A	G/G	C5	34,459,111	G/G	A/A	C6	36,993,630	A/A	G/G	C7	42,330,188	G/G	A/A	C8	33,982,312	G/G	A/A	C9	35,333,860	C/C	A/A
C1	24,451,849	T/T	C/C	C2	22,880,428	A/A	G/G	C3	10,885,445	G/G	C/C	C3	57,312,611	T/T	C/C	C4	51,454,862	C/C	G/G	C5	34,641,885	A/A	C/C	C6	36,993,639	G/G	A/A	C7	42,330,210	T/T	C/C	C8	33,982,349	C/C	G/G	C9	35,333,980	T/T	C/C
C1	24,493,949	T/T	G/G	C2	22,882,792	G/G	A/A	C3	10,885,464	G/G	T/T	C3	57,312,618	A/A	T/T	C4	51,464,110	G/G	C/C	C5	34,900,507	A/A	T/T	C6	37,129,818	C/C	T/T	C7	42,338,830	C/C	T/T	C8	34,003,122	C/C	T/T	C9	35,334,007	G/G	A/A
C1	24,494,044	A/A	G/G	C2	23,130,580	C/C	T/T	C3	11,303,616	T/T	G/G	C3	57,630,392	T/T	C/C	C4	51,465,296	T/T	C/C	C5	34,930,631	T/T	C/C	C6	37,136,670	G/G	T/T	C7	42,396,051	T/T	C/C	C8	34,019,125	C/C	A/A	C9	35,334,079	C/C	T/T
C1	24,500,753	A/A	G/G	C2	23,130,591	C/C	A/A	C3	11,335,368	G/G	C/C	C3	57,630,588	T/T	C/C	C4	51,465,302	C/C	T/T	C5	34,947,628	G/G	A/A	C6	37,137,145	T/T	G/G	C7	42,396,053	A/A	C/C	C8	34,025,457	A/A	G/G	C9	35,399,660	T/T	C/C
C1	24,535,695	A/A	C/C	C2	23,130,651	C/C	T/T	C3	11,335,444	C/C	G/G	C3	57,672,898	A/A	C/C	C4	51,533,624	T/T	A/A	C5	34,962,589	C/C	G/G	C6	37,250,277	C/C	G/G	C7	42,587,537	T/T	C/C	C8	34,048,193	G/G	C/C	C9	35,399,706	C/C	G/G
C1	24,535,813	A/A	G/G	C2	23,130,690	G/G	T/T	C3	11,650,257	T/T	C/C	C3	57,870,724	T/T	C/C	C4	51,533,645	A/A	G/G	C5	35,303,693	A/A	T/T	C6	37,265,983	A/A	G/G	C7	42,602,533	G/G	T/T	C8	34,048,200	T/T	C/C	C9	35,453,157	C/C	T/T
C1	24,535,839	C/C	A/A	C2	23,130,700	G/G	C/C	C3	11,651,517	A/A	G/G	C3	57,926,687	A/A	G/G	C4	51,533,672	C/C	A/A	C5	35,305,466	T/T	C/C	C6	37,293,244	A/A	C/C	C7	42,603,503	G/G	T/T	C8	34,048,361	C/C	T/T	C9	35,453,170	G/G	A/A
C1	24,614,402	C/C	T/T	C2	23,785,093	A/A	T/T	C3	11,668,326	T/T	C/C	C3	58,182,581	A/A	G/G	C4	51,540,314	A/A	T/T	C5	35,438,889	T/T	C/C	C6	37,293,268	G/G	T/T	C7	42,603,523	A/A	T/T	C8	34,058,082	C/C	G/G	C9	35,453,178	C/C	T/T
C1	24,614,546	G/G	C/C	C2	23,788,002	C/C	A/A	C3	11,668,332	T/T	C/C	C3	58,193,621	G/G	A/A	C4	51,564,157	C/C	A/A	C5	35,438,895	T/T	G/G	C6	37,293,417	C/C	T/T	C7	42,605,434	A/A	T/T	C8	34,058,136	C/C	G/G	C9	35,453,227	C/C	A/A
C1	24,614,552	A/A	C/C	C2	23,828,517	C/C	T/T	C3	11,668,338	G/G	A/A	C3	58,250,112	C/C	T/T	C4	51,565,160	G/G	A/A	C5	35,489,855	T/T	A/A	C6	37,293,682	A/A	G/G	C7	42,666,101	A/A	C/C	C8	34,061,036	A/A	G/G	C9	35,686,738	A/A	G/G
C1	24,652,936	C/C	A/A	C2	24,010,384	C/C	T/T	C3	11,674,465	A/A	C/C	C3	58,250,115	A/A	G/G	C4	51,565,233	G/G	T/T	C5	36,167,381	C/C	T/T	C6	37,446,539	A/A													

Appendix III *Cont.***Table S3: *Cont.* : Differences in Allelic variants at various chromosomes positions in parent lines; the cultivated rapid cycling DHSL150 and C07007.**

Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007				
C1	27,243,137	T/T	C/C	C2	26,341,752	A/A	C/C	C3	12,534,868	C/C	G/G	C3	60,182,246	C/C	A/A	C4	51,781,057	C/C	A/A	C5	38,045,625	C/C	G/G	C6	37,961,371	A/A	T/T	C7	43,625,850	C/C	T/T	C8	34,793,805	A/A	G/G	C9	36,362,999	T/T	A/A
C1	27,294,390	T/T	G/G	C2	26,341,764	A/A	G/G	C3	12,575,203	T/T	G/G	C3	60,189,670	T/T	C/C	C4	51,989,235	G/G	C/C	C5	38,154,468	G/G	A/A	C6	37,975,130	C/C	T/T	C7	43,625,853	T/T	C/C	C8	34,826,449	A/A	T/T	C9	36,430,562	A/A	G/G
C1	27,312,525	T/T	C/C	C2	26,419,980	G/G	A/A	C3	13,075,568	C/C	T/T	C3	60,272,327	A/A	T/T	C4	52,063,517	G/G	A/A	C5	38,669,513	C/C	T/T	C6	37,975,142	C/C	T/T	C7	43,752,330	T/T	C/C	C8	35,139,944	C/C	T/T	C9	36,513,135	C/C	G/G
C1	27,312,553	G/G	A/A	C2	26,544,711	A/A	G/G	C3	13,087,937	A/A	C/C	C3	60,272,339	A/A	T/T	C4	52,063,550	A/A	G/G	C5	38,669,609	C/C	A/A	C6	37,998,814	G/G	A/A	C7	43,760,423	A/A	G/G	C8	35,139,973	A/A	G/G	C9	36,563,650	T/T	G/G
C1	27,525,788	A/A	G/G	C2	26,577,215	C/C	A/A	C3	13,230,633	C/C	G/G	C3	60,400,035	T/T	G/G	C4	52,167,717	G/G	A/A	C5	38,669,650	T/T	A/A	C6	38,011,090	C/C	T/T	C7	43,778,880	C/C	T/T	C8	35,238,965	T/T	A/A	C9	36,564,271	A/A	G/G
C1	27,525,805	T/T	C/C	C2	26,737,783	A/A	G/G	C3	13,230,665	T/T	C/C	C3	60,474,786	G/G	C/C	C4	52,237,453	C/C	T/T	C5	38,670,714	T/T	A/A	C6	38,012,471	G/G	A/A	C7	43,800,223	C/C	T/T	C8	35,239,841	T/T	C/C	C9	36,565,248	G/G	A/A
C1	27,525,807	G/G	A/A	C2	26,737,838	G/G	A/A	C3	13,232,989	T/T	C/C	C3	60,518,901	A/A	C/C	C4	52,237,465	G/G	A/A	C5	38,697,253	G/G	T/T	C6	38,040,024	T/T	C/C	C7	43,801,117	C/C	G/G	C8	35,292,120	T/T	G/G	C9	36,565,272	C/C	G/G
C1	27,527,711	G/G	A/A	C2	26,845,166	G/G	C/C	C3	13,423,908	A/A	G/G	C3	60,533,631	A/A	C/C	C4	52,280,580	A/A	T/T	C5	38,697,255	T/T	A/A	C6	38,179,045	A/A	C/C	C7	43,802,405	C/C	G/G	C8	35,292,162	G/G	C/C	C9	36,565,308	A/A	C/C
C1	27,527,717	A/A	T/T	C2	26,845,206	C/C	T/T	C3	13,423,946	G/G	A/A	C3	60,557,115	T/T	C/C	C4	52,290,446	T/T	C/C	C5	38,710,255	G/G	T/T	C6	38,242,903	T/T	A/A	C7	43,803,996	A/A	G/G	C8	35,292,194	C/C	T/T	C9	36,565,347	G/G	A/A
C1	27,582,913	A/A	G/G	C2	26,935,255	G/G	T/T	C3	13,517,279	G/G	A/A	C3	60,557,177	C/C	T/T	C4	52,317,325	G/G	T/T	C5	38,710,303	T/T	C/C	C6	38,270,278	A/A	C/C	C7	43,804,011	G/G	A/A	C8	35,443,100	C/C	G/G	C9	36,565,374	G/G	A/A
C1	27,582,969	A/A	T/T	C2	26,935,300	G/G	A/A	C3	13,517,370	A/A	G/G	C3	60,619,557	A/A	G/G	C4	52,434,009	G/G	A/A	C5	38,710,348	A/A	G/G	C6	38,270,287	G/G	A/A	C7	43,804,083	A/A	G/G	C8	35,699,762	G/G	A/A	C9	36,565,451	A/A	G/G
C1	27,582,990	A/A	G/G	C2	26,959,971	A/A	T/T	C3	13,530,174	A/A	T/T	C3	60,619,726	A/A	G/G	C4	52,528,145	T/T	A/A	C5	38,735,650	T/T	A/A	C6	38,270,320	T/T	C/C	C7	43,804,261	C/C	T/T	C8	35,780,462	A/A	T/T	C9	36,619,935	G/G	A/A
C1	27,582,991	T/T	A/A	C2	26,993,705	A/A	G/G	C3	13,564,061	C/C	T/T	C3	60,619,733	T/T	C/C	C4	52,528,147	T/T	C/C	C5	38,735,665	G/G	A/A	C6	38,298,703	T/T	C/C	C7	43,907,587	G/G	C/C	C8	35,780,485	A/A	G/G	C9	36,620,044	C/C	T/T
C1	27,691,339	G/G	T/T	C2	27,062,250	C/C	G/G	C3	13,564,086	C/C	T/T	C3	60,789,250	T/T	C/C	C4	52,528,179	C/C	G/G	C5	38,735,835	T/T	C/C	C6	38,376,942	A/A	G/G	C7	44,016,740	T/T	A/A	C8	35,797,152	G/G	T/T	C9	37,443,430	C/C	G/G
C1	27,728,326	G/G	T/T	C2	27,065,488	T/T	C/C	C3	13,567,915	G/G	C/C	C3	61,071,972	C/C	T/T	C4	52,547,849	T/T	C/C	C5	38,756,170	G/G	A/A	C6	38,377,067	T/T	G/G	C7	44,017,885	A/A	C/C	C8	35,811,711	A/A	T/T	C9	37,443,442	C/C	G/G
C1	27,728,327	T/T	C/C	C2	27,065,854	A/A	G/G	C3	13,632,028	G/G	T/T	C3	61,072,845	C/C	G/G	C4	52,653,014	G/G	A/A	C5	38,756,235	C/C	G/G	C6	38,471,239	A/A	G/G	C7	44,017,897	A/A	C/C	C8	35,811,902	G/G	A/A	C9	37,443,537	A/A	G/G
C1	27,730,083	G/G	A/A	C2	27,080,003	A/A	C/C	C3	13,632,094	G/G	T/T	C3	61,104,183	A/A	G/G	C4	52,736,649	A/A	C/C	C5	38,756,252	G/G	C/C	C6	38,490,228	A/A	T/T	C7	44,018,025	T/T	C/C	C8	35,821,843	A/A	G/G	C9	37,950,123	G/G	A/A
C1	27,730,113	T/T	C/C	C2	27,090,712	G/G	A/A	C3	13,635,579	G/G	T/T	C3	61,104,807	G/G	T/T	C4	52,736,913	A/A	G/G	C5	38,756,264	G/G	A/A	C6	38,493,446	T/T	C/C	C7	44,064,358	C/C	T/T	C8	35,822,003	C/C	T/T	C9	37,963,560	T/T	A/A
C1	27,850,509	T/T	C/C	C2	27,090,773	T/T	C/C	C3	13,761,592	C/C	G/G	C3	61,107,139	A/A	G/G	C4	52,736,937	T/T	C/C	C5	38,758,455	G/G	A/A	C6	38,493,540	C/C	T/T	C7	44,064,364	C/C	T/T	C8	35,847,672	C/C	G/G	C9	37,993,808	C/C	A/A
C1	28,167,458	T/T	A/A	C2	27,156,289	C/C	T/T	C3	13,761,638	G/G	A/A	C3	61,107,162	G/G	A/A	C4	52,736,998	C/C	T/T	C5	38,790,210	A/A	G/G	C6	38,493,579	A/A	G/G	C7	44,065,012	G/G	T/T	C8	35,905,876	A/A	T/T	C9	38,007,893	G/G	C/C
C1	28,167,467	T/T	A/A	C2	27,326,653	T/T	C/C	C3	13,761,644	G/G	T/T	C3	61,107,170	G/G	A/A	C4	52,737,071	G/G	A/A	C5	38,794,775	A/A	G/G	C6	38,499,440	T/T	G/G	C7	44,274,649	C/C	A/A	C8	35,946,606	C/C	G/G	C9	38,012,201	A/A	C/C
C1	28,167,523	T/T	A/A	C2	27,326,723	A/A	T/T	C3	13,762,449	A/A	G/G	C3	61,195,785	T/T	A/A	C4	52,763,057	C/C	T/T	C5	38,794,940	C/C	A/A	C6	38,499,455	T/T	C/C	C7	44,333,265	G/G	A/A	C8	36,105,111	T/T	C/C	C9	38,110,773	T/T	A/A
C1	28,168,481	T/T	C/C	C2	27,326,745	G/G	T/T	C3	13,768,044	A/A	G/G	C3	61,401,492	T/T	C/C	C4	52,883,168	T/T	C/C	C5	38,951,768	C/C	A/A	C6	38,499,516	G/G	C/C	C7	44,333,362	A/A	C/C	C8	36,319,656	T/T	A/A	C9	38,110,775	T/T	A/A
C1	28,173,927	T/T	A/A	C2	27,390,255	T/T	C/C	C3	13,768,158	C/C	A/A	C3	61,447,012	G/G	A/A	C4	52,892,280	G/G	A/A	C5	38,951,805	T/T	A/A	C6	38,499,536	C/C	T/T	C7	44,466,318	T/T	C/C	C8	36,326,658	G/G	A/A	C9	38,110,917	T/T	C/C
C1	28,263,245	C/C	T/T	C2	27,562,361	G/G	T/T	C3	13,804,969	G/G	A/A	C3	61,447,039	A/A	G/G	C4	52,892,436	G/G	C/C	C5	38,951,912	T/T	G/G	C6	38,499,540	C/C	G/G	C7	44,498,760	G/G	T/T	C8	36,548,677	T/T	C/C	C9	38,153,542	A/A	G/G
C1	28,263,281	A/A	C/C	C2	27,562,487	T/T	G/G	C3	13,804,975	G/G	A/A	C3	61,447,088	G/G	C/C	C4	52,905,413	G/G	A/A	C5	38,952,153	C/C	A/A	C6	38,503,412	T/T	C/C	C7	44,558,338	G/G	A/A	C8	36,555,478	G/G	A/A	C9	38,155,044	G/G	T/T
C1	28,512,464	C/C	T/T	C2	27,570,684	T/T	G/G	C3	13,853,766	C/C	A/A	C3	61,447,089	T/T	A/A	C4	52,905,431	C/C	A/A	C5	38,952,584	G/G	A/A	C6	38,510,381	G/G	T/T	C7	44,647,788	G/G	A/A	C8	36,555,490	T/T	C/C	C9	38,256,615	C/C	T/T
C1	28,527,832	C/C	G/G	C2	27,696,466	C/C	T/T	C3	13,931,115	C/C	T/T	C3	61,447,107	C/C	G/G	C4	52,905,440	G/G	T/T	C5	38,952,657	A/A	T/T	C6	38,510,469	A/A	T/T	C7	44,647,797	T/T	G/G	C8	36,622,201	A/A	G/G	C9	38,256,839	A/A	C/C
C1	28,527,834	A/A	C/C	C2	27,696,617	T/T	G/G	C3	13,993,737	T/T	G/G	C3	61,481,193	T/T	G/G	C4	52,978,145	C/C	T/T	C5	38,952,889	A/A	G/G	C6	38,515,572	G/G	T/T	C7	44,647,821	C/C	T/T	C8	36,681,006	A/A	G/G	C9	38,329,367	C/C	T/T
C1	28,527,851	A/A	C/C	C2	27,698,824	T/T	G/G	C3	13,993,832	C/C	T/T	C3	61,481,245	T/T	A/A	C4	52,985,744	A/A	C/C	C5	38,953,773	A/A	T/T	C6	38,515,620	G/G	A/A	C7	44,647,824	T/T	C/C	C8	36,702,481	T/T	C/C	C9	38,329,379	T/T	C/C
C1	28,592,689	A/A	T/T	C2	27,956,167	G/G	T/T	C3	13,994,061	A/A	T/T	C3	61,643,326	G/G	A/A	C4	52,997,717	A/A	G/G	C5	38,987,082	G/G	A/A	C6	38,515,623	A/A	T/T	C7	44,647,905	A/A	T/T	C8	36,702,503	A/A	G/G	C9	38,329,444	A/A	T/T
C1	28,592,763	A/A	G/G	C2	27,962,371	T/T	A/A	C3	14,083,611	G/G	A/A	C3	61,688,296	C/C	T/T	C4	52,997,726	G/G	A/A	C5	38,987,146	G/G	A/A	C6	38,600,672	T/T	C/C	C7	44,648,769	C/C	G/G	C8	36,775,711	G/G	T/T	C9	38,329,460	C/C	T/T
C1	28,592,804	C/C	A/A	C2	27,963,636	A/A	T/T	C3	14,178,085	A/A	C/C	C3	61,740,559	A/A	G/G	C4	52,997,744	C/C	A/A	C5	38,989,663	T/T	G/G	C6	38,601,265	T/T	G/G	C7	44,648,801	C/C	T/T	C8	36,775,830	A/A	T/T	C9	38,343,335	G/G	T/T
C1	28,738,109	C/C	G/G	C2	28,272,017	T/T	C/C	C3	14,178,867	G/G	A/A	C3	61,745,991	G/G	A/A	C4	52,997,747	C/C	T/T	C5	39,104,134	G/G	C/C	C6	38,601,516	G/G	A/A	C7	44,648,820	C/C	T/T	C8	36,779,730	C/C	T/T	C9	38,351,411	A/A	G/G
C1	28,897,939	T/T	C/C	C2	28,413,869	G/G	A/A	C3	14,334,743	A/A	G/G	C3	61,761,006	T/T	A/A	C4	52,997,881	G/G	C/C	C5	39,135,148	G/G	C/C	C6	38,601,576	C/C	G/G	C7	44,715,233	C/C	T/T	C8	36,790,567	T/T	C/C	C9	38,373,812	T/T	A/A
C1	29,047,811	A/A	G/G	C2	28,463,859	T/T	A/A	C3	14,334,809	G/G	T/T	C3	61,769,366	G/G	A/A	C4	53,001,634	T/T	A/A	C5	39,135,167																		

Appendix III *Cont.***Table S3: *Cont.* :** Differences in Allelic variants at various chromosomes positions in parent lines; the cultivated rapid cycling DHSL150 and C07007.

Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007								
C1	30,872,841	G/G	A/A	C2	29,418,206	G/G	T/T	C3	14,823,567	T/T	C/C	C3	62,312,888	C/C	T/T	C4	53,410,018	G/G	C/C	C5	39,650,791	T/T	C/C	C6	38,929,206	C/C	T/T	C7	45,314,993	C/C	A/A	C8	36,979,264	A/A	T/T	C9	39,325,484	C/C	G/G
C1	30,872,862	A/A	C/C	C2	29,442,051	G/G	T/T	C3	15,126,260	C/C	T/T	C3	62,403,350	T/T	G/G	C4	53,410,081	C/C	T/T	C5	39,653,125	C/C	A/A	C6	38,929,229	T/T	G/G	C7	45,315,069	C/C	T/T	C8	36,979,291	T/T	G/G	C9	39,325,701	C/C	G/G
C1	30,918,194	G/G	A/A	C2	29,442,062	C/C	T/T	C3	15,205,433	A/A	G/G	C3	62,434,364	G/G	A/A	C4	53,410,127	A/A	G/G	C5	39,653,176	G/G	A/A	C6	38,929,230	T/T	G/G	C7	45,315,079	A/A	C/C	C8	36,979,368	A/A	G/G	C9	39,327,866	T/T	C/C
C1	30,918,202	C/C	A/A	C2	29,476,573	C/C	G/G	C3	15,213,725	A/A	T/T	C3	62,557,276	C/C	A/A	C4	53,410,139	C/C	G/G	C5	39,653,201	C/C	T/T	C6	38,970,934	C/C	T/T	C7	45,324,946	C/C	T/T	C8	37,090,988	A/A	C/C	C9	39,374,361	T/T	G/G
C1	30,919,232	T/T	C/C	C2	29,479,840	A/A	C/C	C3	15,213,757	G/G	T/T	C3	62,726,074	A/A	C/C	C4	53,410,160	T/T	G/G	C5	39,653,981	A/A	C/C	C6	38,970,937	T/T	A/A	C7	45,356,653	A/A	C/C	C8	37,093,747	T/T	C/C	C9	39,481,410	A/A	T/T
C1	30,938,869	A/A	C/C	C2	29,479,900	A/A	T/T	C3	15,215,178	T/T	G/G	C3	62,747,359	A/A	G/G	C4	53,413,859	G/G	C/C	C5	39,654,024	G/G	T/T	C6	39,011,798	C/C	G/G	C7	45,372,570	A/A	T/T	C8	37,097,121	T/T	C/C	C9	39,494,038	A/A	G/G
C1	31,228,819	A/A	T/T	C2	29,582,860	T/T	C/C	C3	15,374,721	A/A	G/G	C3	62,747,366	A/A	G/G	C4	53,413,876	C/C	A/A	C5	39,665,138	T/T	C/C	C6	39,076,565	T/T	C/C	C7	45,372,594	G/G	A/A	C8	37,112,324	C/C	T/T	C9	39,494,065	T/T	C/C
C1	31,469,357	C/C	T/T	C2	29,594,110	G/G	C/C	C3	15,374,728	A/A	G/G	C3	62,747,392	T/T	C/C	C4	53,509,035	A/A	G/G	C5	39,665,252	G/G	T/T	C6	39,175,149	C/C	A/A	C7	45,372,677	A/A	C/C	C8	37,112,327	G/G	A/A	C9	39,495,100	C/C	A/A
C1	31,556,646	T/T	C/C	C2	29,679,960	C/C	T/T	C3	15,440,142	C/C	T/T	C3	63,127,865	C/C	T/T	C5	20,511	C/C	A/A	C5	39,665,361	G/G	A/A	C8	39,181,726	T/T	C/C	C7	45,517,339	T/T	C/C	C8	37,115,232	C/C	A/A	C9	39,684,159	A/A	G/G
C1	31,619,423	T/T	C/C	C2	29,761,345	G/G	A/A	C3	15,440,163	C/C	G/G	C3	63,144,980	G/G	A/A	C5	31,899	C/C	A/A	C5	39,665,363	T/T	C/C	C6	39,181,756	A/A	G/G	C7	45,522,403	C/C	T/T	C8	37,115,310	G/G	T/T	C9	39,716,319	C/C	T/T
C1	31,773,323	A/A	G/G	C2	29,761,375	T/T	G/G	C3	15,440,177	C/C	T/T	C3	63,146,499	T/T	G/G	C5	32,341	A/A	T/T	C5	39,668,258	T/T	G/G	C6	39,181,779	G/G	A/A	C7	45,522,409	T/T	C/C	C8	37,175,256	C/C	T/T	C9	39,716,321	T/T	A/A
C1	31,885,119	G/G	T/T	C2	29,918,525	C/C	T/T	C3	15,441,767	G/G	A/A	C3	63,146,523	T/T	G/G	C5	48,605	C/C	T/T	C5	39,668,305	A/A	G/G	C6	39,181,783	G/G	A/A	C7	45,526,487	T/T	C/C	C8	37,178,399	A/A	G/G	C9	40,393,653	G/G	A/A
C1	32,013,556	C/C	G/G	C2	29,918,543	T/T	A/A	C3	15,445,314	C/C	T/T	C3	63,254,600	C/C	T/T	C5	68,024	G/G	T/T	C5	39,677,888	G/G	C/C	C6	39,334,135	T/T	A/A	C7	45,526,509	C/C	A/A	C8	37,212,750	T/T	C/C	C9	40,871,872	A/A	C/C
C1	32,120,843	T/T	C/C	C2	29,918,544	A/A	T/T	C3	15,445,326	G/G	T/T	C3	63,279,489	T/T	A/A	C5	68,033	T/T	C/C	C5	39,677,904	C/C	A/A	C6	39,334,339	G/G	T/T	C7	45,526,518	T/T	A/A	C8	37,253,053	A/A	G/G	C9	41,071,412	C/C	T/T
C1	32,160,094	C/C	G/G	C2	29,920,010	G/G	A/A	C3	15,445,636	T/T	C/C	C3	63,484,123	C/C	T/T	C5	68,238	T/T	C/C	C5	39,677,928	G/G	A/A	C6	39,350,210	G/G	T/T	C7	45,538,728	G/G	A/A	C8	37,264,033	C/C	T/T	C9	41,177,285	C/C	T/T
C1	32,165,513	G/G	C/C	C2	29,957,621	C/C	A/A	C3	15,445,649	T/T	G/G	C3	63,524,550	T/T	C/C	C5	85,755	G/G	A/A	C5	39,678,018	A/A	G/G	C6	39,350,354	A/A	T/T	C7	45,569,054	A/A	C/C	C8	37,264,070	C/C	T/T	C9	41,215,500	A/A	G/G
C1	32,227,656	A/A	C/C	C2	29,957,770	A/A	C/C	C3	15,520,327	T/T	A/A	C3	63,524,574	T/T	C/C	C5	85,759	G/G	C/C	C5	39,681,948	T/T	C/C	C6	39,350,373	A/A	C/C	C7	45,788,775	A/A	T/T	C8	37,387,783	T/T	A/A	C9	41,215,502	G/G	A/A
C1	32,692,669	A/A	T/T	C2	29,986,937	C/C	A/A	C3	15,547,891	A/A	C/C	C3	63,567,265	A/A	G/G	C5	85,769	A/A	G/G	C5	39,882,126	A/A	G/G	C6	39,407,586	A/A	C7	45,869,400	A/A	G/G	C8	37,387,792	A/A	G/G	C9	41,576,103	T/T	C/C	
C1	32,752,455	A/A	G/G	C2	29,986,965	C/C	G/G	C3	15,547,936	A/A	G/G	C3	63,575,109	G/G	A/A	C5	342,522	T/T	G/G	C5	39,882,142	A/A	G/G	C6	39,412,291	T/T	A/A	C7	45,902,609	T/T	C/C	C8	37,466,502	G/G	C/C	C9	41,576,154	G/G	T/T
C1	33,081,235	A/A	C/C	C2	30,152,341	T/T	C/C	C3	15,585,578	C/C	G/G	C3	63,973,183	C/C	T/T	C5	364,139	G/G	T/T	C5	39,882,181	G/G	A/A	C6	39,420,626	G/G	A/A	C7	45,902,734	T/T	C/C	C8	37,519,184	T/T	C/C	C9	41,581,320	C/C	A/A
C1	33,157,769	T/T	G/G	C2	30,158,145	G/G	A/A	C3	15,890,671	A/A	G/G	C3	63,973,327	T/T	G/G	C5	364,936	T/T	A/A	C5	39,882,199	T/T	A/A	C6	39,420,629	A/A	G/G	C7	45,902,786	G/G	A/A	C8	37,519,244	T/T	C/C	C9	41,581,330	A/A	C/C
C1	33,225,063	C/C	T/T	C2	30,169,855	C/C	T/T	C3	15,892,778	G/G	T/T	C3	63,978,478	A/A	T/T	C5	365,000	G/G	A/A	C5	39,882,226	A/A	G/G	C6	39,427,438	T/T	C/C	C7	45,911,331	C/C	T/T	C8	37,539,148	C/C	T/T	C9	41,593,495	T/T	C/C
C1	33,366,353	T/T	G/G	C2	30,264,529	T/T	G/G	C3	15,902,045	T/T	C/C	C3	64,623,092	T/T	A/A	C5	365,012	C/C	A/A	C5	39,916,907	G/G	T/T	C6	39,450,543	T/T	C/C	C7	45,922,757	A/A	G/G	C8	37,539,217	A/A	G/G	C9	41,593,515	G/G	A/A
C1	33,389,147	T/T	A/A	C2	30,282,279	A/A	G/G	C3	15,902,136	T/T	C/C	C3	64,624,029	T/T	C/C	C5	378,294	G/G	C/C	C5	39,916,920	T/T	G/G	C6	39,453,702	T/T	G/G	C7	46,033,658	G/G	T/T	C8	37,539,543	T/T	C/C	C9	41,593,716	C/C	T/T
C1	33,433,767	G/G	A/A	C2	30,483,515	T/T	C/C	C3	15,925,016	C/C	G/G	C3	64,627,141	T/T	C/C	C5	378,448	C/C	T/T	C5	39,919,197	C/C	T/T	C6	39,461,562	A/A	G/G	C7	46,049,965	G/G	A/A	C8	37,649,187	T/T	G/G	C9	41,620,509	T/T	A/A
C1	33,433,904	G/G	T/T	C2	30,483,523	A/A	T/T	C3	15,929,306	A/A	G/G	C3	64,627,253	C/C	T/T	C5	378,612	G/G	A/A	C5	39,919,227	G/G	T/T	C6	39,594,005	C/C	T/T	C7	46,050,086	A/A	G/G	C8	37,687,680	T/T	C/C	C9	41,621,867	C/C	T/T
C1	33,480,843	T/T	C/C	C2	30,762,412	G/G	A/A	C3	15,954,112	T/T	G/G	C3	64,642,639	A/A	T/T	C5	383,514	G/G	C/C	C5	39,922,678	A/A	G/G	C7	152,990	T/T	A/A	C7	46,068,962	C/C	G/G	C8	37,687,686	T/T	C/C	C9	41,623,138	C/C	G/G
C1	33,496,161	C/C	T/T	C2	30,762,426	G/G	A/A	C3	15,986,115	A/A	G/G	C3	64,647,174	A/A	G/G	C5	408,243	A/A	T/T	C5	39,922,704	A/A	C/C	C7	154,060	G/G	A/A	C7	46,068,974	C/C	T/T	C8	37,687,701	C/C	G/G	C9	41,623,211	C/C	T/T
C1	33,521,142	C/C	G/G	C2	30,762,495	T/T	C/C	C3	16,061,792	C/C	T/T	C3	64,647,270	G/G	C/C	C5	409,891	C/C	T/T	C5	39,922,706	C/C	T/T	C7	172,388	A/A	T/T	C7	46,069,112	C/C	T/T	C8	37,689,626	G/G	C/C	C9	41,987,796	T/T	C/C
C1	33,521,308	T/T	A/A	C2	30,783,992	C/C	A/A	C3	16,128,767	T/T	C/C	C3	64,791,856	T/T	C/C	C5	409,902	G/G	A/A	C5	39,922,720	G/G	A/A	C7	195,304	A/A	C/C	C7	46,069,139	T/T	C/C	C8	37,712,889	A/A	G/G	C9	42,828,194	A/A	C/C
C1	33,541,256	C/C	A/A	C2	30,783,993	A/A	G/G	C3	16,280,950	T/T	C/C	C3	64,791,891	C/C	T/T	C5	420,178	G/G	A/A	C5	40,048,748	C/C	A/A	C7	265,937	A/A	G/G	C7	46,072,115	G/G	T/T	C8	37,719,247	C/C	G/G	C9	42,828,236	A/A	C/C
C1	33,541,267	G/G	A/A	C2	31,230,130	T/T	C/C	C3	16,281,126	C/C	T/T	C3	64,791,912	T/T	A/A	C5	420,241	T/T	C/C	C5	40,142,933	A/A	G/G	C7	327,063	G/G	A/A	C7	46,106,063	C/C	T/T	C8	37,764,351	G/G	A/A	C9	42,866,861	C/C	T/T
C1	33,541,714	G/G	A/A	C2	31,230,162	G/G	A/A	C3	16,281,143	A/A	G/G	C3	64,816,599	C/C	T/T	C5	450,003	A/A	G/G	C5	40,143,120	T/T	G/G	C7	445,998	C/C	G/G	C7	46,121,860	G/G	A/A	C8	37,796,251	C/C	A/A	C9	42,867,097	A/A	G/G
C1	33,608,850	G/G	C/C	C2	31,230,169	G/G	A/A	C3	16,296,008	C/C	A/A	C3	64,843,503	C/C	G/G	C5	450,043	A/A	C/C	C5	40,150,516	T/T	C/C	C7	446,009	G/G	A/A	C7	46,121,916	G/G	A/A	C8	37,797,695	G/G	C/C	C9	43,185,898	A/A	G/G
C1	33,636,822	G/G	A/A	C2	31,230,250	G/G	C/C	C3	16,436,167	C/C	T/T	C3	64,843,517	C/C	G/G	C5	461,003	A/A	C/C	C5	40,451,925	T/T	G/G	C7	452,080	A/A	G/G	C7	46,176,377	T/T	C/C	C8	37,797,714	G/G	A/A	C9	43,196,362	C/C	T/T
C1	33,869,072	T/T	C/C	C2	31,282,994	C/C	A/A	C3	16,436,211	C/C	G/G	C3	64,860,077	G/G	A/A	C5	461,038	T/T	C/C	C5	40,570,121	C/C	T/T	C7	452,099	G/G	T/T	C7	46,176,402	T/T	C/C	C8	37,797,746	A/A	C/C	C9	43,215,334	G/G	A/A
C1	34,154																																						

Appendix III *Cont.***Table S3: *Cont.* : Differences in Allelic variants at various chromosomes positions in parent lines; the cultivated rapid cycling DHSL150 and C07007.**

Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007								
C1	34,322,718	T/T	C/C	C2	32,289,609	C/C	A/A	C3	17,006,470	T/T	C/C	C4	693,595	T/T	C/C	C5	803,560	C/C	G/G	C5	41,965,913	A/A	T/T	C7	2,734,139	A/A	G/G	C7	46,700,897	T/T	A/A	C8	38,429,406	T/T	C/C	C9	44,890,829	T/T	C/C
C1	34,322,725	C/C	A/A	C2	32,289,610	A/A	T/T	C3	17,006,492	G/G	A/A	C4	693,623	A/A	T/T	C5	803,608	A/A	C/C	C5	42,144,863	T/T	A/A	C7	2,890,467	C/C	G/G	C7	46,962,030	A/A	G/G	C8	38,429,505	G/G	A/A	C9	45,143,152	T/T	G/G
C1	34,322,920	A/A	T/T	C2	32,738,921	A/A	C/C	C3	17,006,497	T/T	G/G	C4	693,627	A/A	G/G	C5	807,665	C/C	A/A	C5	42,152,432	C/C	T/T	C7	2,890,511	G/G	T/T	C7	46,962,167	C/C	T/T	C8	38,435,779	G/G	C/C	C9	45,146,862	T/T	C/C
C1	34,323,024	G/G	T/T	C2	32,739,020	G/G	T/T	C3	17,006,550	C/C	T/T	C4	694,016	G/G	C/C	C5	830,213	A/A	G/G	C5	42,204,939	C/C	T/T	C7	2,917,198	C/C	T/T	C7	46,971,673	A/A	G/G	C8	38,436,022	C/C	T/T	C9	45,146,889	G/G	A/A
C1	34,354,043	T/T	C/C	C2	32,745,941	A/A	C/C	C3	17,042,702	G/G	T/T	C4	935,530	A/A	G/G	C5	873,434	G/G	A/A	C5	42,204,945	C/C	T/T	C7	2,917,212	C/C	A/A	C7	46,971,716	T/T	C/C	C8	38,462,473	G/G	T/T	C9	45,147,021	A/A	C/C
C1	34,354,072	T/T	C/C	C2	32,746,070	C/C	T/T	C3	17,074,245	A/A	G/G	C4	939,560	A/A	G/G	C5	873,541	G/G	T/T	C5	42,205,046	T/T	A/A	C7	2,958,401	G/G	A/A	C7	46,991,615	C/C	T/T	C8	38,462,512	G/G	C/C	C9	45,393,388	T/T	G/G
C1	34,357,831	C/C	T/T	C2	32,746,811	T/T	C/C	C3	17,132,331	C/C	T/T	C4	964,500	A/A	G/G	C5	902,421	G/G	A/A	C5	42,206,339	A/A	C/C	C7	3,024,088	T/T	C/C	C7	47,016,619	C/C	G/G	C8	38,507,458	G/G	A/A	C9	45,410,724	G/G	A/A
C1	34,382,297	G/G	A/A	C2	32,746,820	A/A	T/T	C3	17,150,642	A/A	G/G	C4	965,478	T/T	G/G	C5	919,596	T/T	A/A	C5	42,206,462	A/A	C/C	C7	3,024,138	G/G	T/T	C7	47,035,181	C/C	T/T	C8	38,507,536	T/T	C/C	C9	45,429,036	A/A	G/G
C1	34,598,335	T/T	G/G	C2	32,813,719	C/A	A/A	C3	17,180,306	A/A	G/G	C4	966,291	G/G	A/A	C5	978,512	T/T	C/C	C5	42,206,490	A/A	T/T	C7	3,542,479	A/A	G/G	C7	47,090,655	G/G	A/A	C8	38,518,491	G/G	A/A	C9	45,512,817	C/C	T/T
C1	34,617,067	G/G	T/T	C2	32,887,701	A/A	T/T	C3	17,180,319	A/A	G/G	C4	1,111,042	G/G	A/A	C5	1,008,216	G/G	C/C	C5	42,209,187	C/C	T/T	C7	3,701,357	G/G	A/A	C7	47,121,669	T/T	A/A	C8	38,518,542	G/G	C/C	C9	45,610,955	T/T	G/G
C1	34,663,313	G/G	A/A	C2	32,926,756	T/T	A/A	C3	17,180,331	G/G	A/A	C4	1,369,050	T/T	C/C	C5	1,008,228	A/A	T/T	C5	42,212,960	G/G	A/A	C7	3,701,995	T/T	C/C	C7	47,147,114	G/G	A/A	C8	38,518,545	T/T	C/C	C9	45,610,976	T/T	C/C
C1	34,673,815	C/C	G/G	C2	32,926,795	C/C	G/G	C3	17,401,069	C/C	A/A	C4	1,381,600	C/C	G/G	C5	1,032,408	T/T	C/C	C5	42,221,926	T/T	C/C	C7	3,725,340	C/C	T/T	C7	47,188,614	C/C	T/T	C8	38,522,993	T/T	C/C	C9	45,610,987	C/C	A/A
C1	34,953,160	T/T	C/C	C2	33,034,189	A/A	G/G	C3	17,401,114	G/G	A/A	C4	1,772,665	T/T	C/C	C5	1,032,520	C/C	A/A	C5	42,232,579	A/A	G/G	C7	3,769,178	A/A	T/T	C7	47,188,626	A/A	G/G	C8	38,543,748	G/G	A/A	C9	45,617,106	C/C	T/T
C1	34,953,180	A/A	C/C	C2	33,050,689	G/G	A/A	C3	17,401,219	G/G	C/C	C4	1,772,678	C/C	G/G	C5	1,032,837	C/C	T/T	C5	42,232,583	T/T	C/C	C7	3,770,934	A/A	C/C	C7	47,253,788	T/T	C/C	C8	38,554,687	A/A	T/T	C9	45,663,464	C/C	G/G
C1	35,041,746	C/C	A/A	C2	33,158,192	G/G	T/T	C3	17,434,531	G/G	T/T	C4	1,824,023	G/G	A/A	C5	1,032,843	G/G	C/C	C5	42,232,630	C/C	T/T	C7	3,772,028	A/A	C/C	C7	47,253,851	A/A	G/G	C8	38,554,755	A/A	T/T	C9	46,066,238	T/T	G/G
C1	35,049,227	T/T	C/C	C2	33,226,552	T/T	A/A	C3	17,608,978	T/T	C/C	C4	1,824,065	T/T	G/G	C5	1,033,088	T/T	C/C	C5	42,242,166	C/C	T/T	C7	4,098,906	C/C	T/T	C7	47,253,878	C/C	T/T	C8	38,560,196	G/G	A/A	C9	46,066,253	C/C	T/T
C1	35,110,638	G/G	A/A	C2	33,226,588	G/G	A/A	C3	17,663,604	C/C	T/T	C4	1,824,071	G/G	A/A	C5	1,033,103	C/C	T/T	C5	42,694,205	A/A	G/G	C7	4,099,223	G/G	A/A	C7	47,253,884	T/T	C/C	C8	38,574,895	C/C	T/T	C9	46,066,360	T/T	A/A
C1	35,110,645	T/T	C/C	C2	33,283,544	G/G	T/T	C3	17,759,043	C/C	T/T	C4	1,824,098	C/C	T/T	C5	1,038,262	A/A	T/T	C5	42,891,943	C/C	G/G	C7	4,216,504	T/T	C/C	C7	47,288,286	G/G	C/C	C8	38,574,985	G/G	A/A	C9	46,106,419	A/A	C/C
C1	35,110,657	T/T	G/G	C2	33,788,837	C/C	A/A	C3	17,759,101	G/G	A/A	C4	2,033,285	A/A	G/G	C5	1,043,594	A/A	T/T	C5	42,893,103	G/G	A/A	C7	4,386,136	G/G	T/T	C7	47,288,427	C/C	T/T	C8	38,574,988	G/G	C/C	C9	46,106,433	A/A	G/G
C1	35,110,838	T/T	A/A	C2	33,943,714	G/G	A/A	C3	17,796,653	A/A	C/C	C4	2,033,372	G/G	A/A	C5	1,098,210	T/T	C/C	C5	42,910,351	T/T	A/A	C7	4,531,455	T/T	C/C	C7	47,289,316	A/A	G/G	C8	38,643,194	G/G	T/T	C9	46,106,719	C/C	T/T
C1	35,760,508	G/G	A/A	C2	34,034,736	A/A	G/G	C3	17,796,684	G/G	C/C	C4	2,033,577	C/C	G/G	C5	1,113,624	C/C	A/A	C5	42,950,591	C/C	G/G	C7	4,531,467	C/C	T/T	C7	47,289,351	A/A	G/G	C8	38,758,146	A/A	T/T	C9	46,106,758	T/T	C/C
C1	35,975,242	A/A	T/T	C2	33,934,115	T/T	G/G	C3	17,796,731	C/C	A/A	C4	2,041,711	A/A	C/C	C5	1,113,781	A/A	G/G	C5	43,004,410	A/A	T/T	C7	4,531,538	G/G	A/A	C7	47,306,464	T/T	C/C	C8	38,758,161	C/C	G/G	C9	46,106,140	A/A	T/T
C1	36,004,031	A/A	G/G	C2	34,095,984	A/A	G/G	C3	17,806,820	A/A	T/T	C4	2,079,072	A/A	T/T	C5	1,116,274	A/A	G/G	C5	43,158,236	T/T	C/C	C7	4,700,073	T/T	C/C	C7	47,317,885	C/C	T/T	C8	38,838,174	A/A	G/G	C9	46,329,310	C/C	T/T
C1	36,004,091	C/C	A/A	C2	34,105,055	G/G	A/A	C3	17,898,580	G/G	A/A	C4	2,180,589	C/C	G/G	C5	1,116,292	A/A	T/T	C5	43,158,260	G/G	A/A	C7	4,700,078	A/A	G/G	C7	47,318,023	A/A	C/C	C8	38,838,261	G/G	C/C	C9	46,417,240	A/A	G/G
C1	36,354,149	C/C	A/A	C2	34,105,067	A/A	G/G	C3	17,913,352	G/G	T/T	C4	2,126,106	A/A	T/T	C5	1,122,106	A/A	G/G	C5	43,239,053	A/A	G/G	C7	4,740,824	C/C	T/T	C7	47,318,069	T/T	C/C	C8	38,838,303	C/C	T/T	C9	46,417,336	A/A	C/C
C1	36,354,272	C/C	T/T	C2	34,119,821	A/A	T/T	C3	17,930,155	C/C	T/T	C4	2,473,871	A/A	T/T	C5	1,139,902	G/G	A/A	C5	43,259,531	T/T	A/A	C7	4,807,042	A/A	T/T	C7	47,425,896	T/T	A/A	C8	38,852,856	A/A	T/T	C9	46,489,380	G/G	A/A
C1	36,415,778	C/C	T/T	C2	34,185,295	G/G	A/A	C3	18,291,762	G/G	T/T	C4	2,486,740	C/C	T/T	C5	1,150,267	G/G	A/A	C5	43,259,543	T/T	C/C	C7	4,855,576	T/T	G/G	C7	47,542,363	A/A	G/G	C8	38,852,944	G/G	A/A	C9	46,522,556	C/C	T/T
C1	36,507,990	G/G	T/T	C2	34,220,849	A/A	G/G	C3	18,321,373	A/A	G/G	C4	2,617,609	G/G	C/C	C5	1,150,273	G/G	T/T	C5	43,301,173	G/G	C/C	C7	4,924,469	T/T	C/C	C7	47,542,369	G/G	C/C	C8	38,860,696	G/G	C/C	C9	46,522,559	T/T	G/G
C1	36,526,324	C/C	T/T	C2	34,612,529	G/G	A/A	C3	18,365,280	A/A	G/G	C4	2,617,683	G/G	A/A	C5	1,150,279	G/G	A/A	C5	43,306,866	T/T	G/G	C7	5,042,698	C/C	T/T	C7	47,630,058	G/G	A/A	C8	38,864,529	A/A	G/G	C9	46,807,780	A/A	C/C
C1	36,594,164	C/C	T/T	C2	34,648,759	C/C	T/T	C3	18,431,373	A/A	G/G	C4	2,726,513	T/T	G/G	C5	1,150,782	C/C	T/T	C5	43,360,981	T/T	C/C	C7	5,128,190	A/A	G/G	C7	47,630,128	T/T	C/C	C8	38,864,532	A/A	G/G	C9	46,807,839	G/G	T/T
C1	36,614,582	G/G	A/A	C2	34,704,701	T/T	A/A	C3	18,450,976	C/C	T/T	C4	2,726,528	G/G	C/C	C5	1,150,830	G/G	A/A	C5	43,366,188	A/A	T/T	C7	5,445,191	C/C	A/A	C7	47,630,130	C/C	T/T	C8	38,897,087	G/G	C/C	C9	46,807,845	A/A	T/T
C1	36,614,585	A/A	G/G	C2	34,704,709	T/T	C/C	C3	18,451,291	A/A	T/T	C4	2,742,166	A/A	T/T	C5	1,150,977	T/T	A/A	C5	43,370,662	C/C	A/A	C7	5,596,887	T/T	C/C	C7	47,630,873	T/T	G/G	C8	38,967,944	A/A	C/C	C9	46,807,952	C/C	T/T
C1	36,617,564	A/A	T/T	C2	35,085,506	C/C	G/G	C3	18,458,392	G/G	A/A	C4	2,787,557	G/G	A/A	C5	1,248,191	C/C	T/T	C5	43,370,790	A/A	G/G	C7	5,596,907	G/G	T/T	C7	47,695,542	A/A	T/T	C8	39,058,659	A/A	T/T	C9	46,964,852	C/C	T/T
C1	36,655,869	T/T	C/C	C2	35,105,884	T/T	A/A	C3	18,483,337	A/A	G/G	C4	2,808,414	T/T	A/A	C5	1,289,719	C/C	T/T	C5	43,472,729	C/C	T/T	C7	5,654,312	T/T	C/C	C7	47,695,651	T/T	C/C	C8	39,397,505	A/A	G/G	C9	47,269,490	T/T	C/C
C1	36,768,057	T/T	A/A	C2	35,106,942	T/T	A/A	C3	18,483,346	A/A	T/T	C4	2,808,564	T/T	C/C	C5	1,311,152	C/C	T/T	C5	43,497,455	A/A	C/C	C7	5,654,943	T/T	A/A	C7	47,751,473	G/G	A/A	C8	39,456,687	T/T	C/C	C9	47,269,493	C/C	G/G
C1	36,830,044	A/A	G/G	C2	35,216,489	T/T	G/G	C3	18,488,010	C/C	T/T	C4	2,808,585	A/A	C/C	C5	1,386,634	A/A	C/C	C5	43,498,082	C/C	T/T	C7	5,655,053	G/G	A/A	C7	47,919,353	C/C	T/T	C8	39,549,365	A/A	G/G	C9	47,269,679	A/A	G/G
C1	37,224,687	G/G																																					

Appendix III *Cont.***Table S3: *Cont.* : Differences in Allelic variants at various chromosomes positions in parent lines; the cultivated rapid cycling DHSL150 and C07007.**

Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007				
C1	38,040,709	T/T	A/A	C2	36,170,872	A/A	G/G	C3	19,447,019	G/G	A/A	C4	3,576,714	A/A	G/G	C5	1,969,618	A/A	G/G	C5	43,810,424	T/T	C/C	C7	7,284,189	G/G	A/A	C7	48,216,638	G/G	A/A	C8	39,906,950	C/C	T/T	C9	48,089,817	T/T	G/G
C1	38,063,722	C/C	T/T	C2	36,253,835	C/C	G/G	C3	19,468,178	G/G	A/A	C4	3,700,993	G/G	A/A	C5	2,190,998	C/C	A/A	C5	43,872,478	C/C	T/T	C7	7,285,665	G/G	A/A	C7	48,216,763	A/A	G/G	C8	39,906,966	C/C	T/T	C9	48,089,844	T/T	C/C
C1	38,063,812	C/C	T/T	C2	36,331,434	T/T	G/G	C3	19,475,980	T/T	C/C	C4	3,701,009	T/T	C/C	C5	2,209,525	C/C	G/G	C5	43,872,483	C/C	A/A	C7	7,285,724	A/A	C/C	C7	48,218,566	C/C	A/A	C8	39,986,218	C/C	T/T	C9	48,229,799	G/G	A/A
C1	38,120,612	C/C	A/A	C2	36,395,021	C/C	G/G	C3	19,476,096	C/C	T/T	C4	3,702,842	G/G	A/A	C5	2,209,552	A/A	G/G	C5	43,908,526	T/T	G/G	C7	7,320,700	T/T	C/C	C7	48,221,718	C/C	T/T	C8	39,986,232	C/C	T/T	C9	48,263,497	A/A	G/G
C1	38,121,210	G/G	T/T	C2	36,395,035	G/G	A/A	C3	19,476,129	T/T	C/C	C4	3,702,857	A/A	T/T	C5	2,211,188	T/T	G/G	C5	43,911,976	G/G	C/C	C7	7,321,470	T/T	A/A	C7	48,221,742	A/A	G/G	C8	39,986,248	T/T	A/A	C9	48,263,502	G/G	A/A
C1	38,121,324	G/G	A/A	C2	36,694,247	G/G	A/A	C3	20,583,745	T/T	C/C	C4	3,753,508	G/G	C/C	C5	2,217,873	C/C	T/T	C5	44,119,823	T/T	C/C	C7	7,331,641	A/A	G/G	C7	48,267,439	A/A	C/C	C8	39,992,899	A/A	C/C	C9	48,457,234	A/A	G/G
C1	38,141,134	A/A	G/G	C2	36,694,273	C/C	A/A	C3	20,591,880	G/G	A/A	C4	3,753,738	C/C	T/T	C5	2,220,914	A/A	G/G	C5	44,235,456	A/A	C/C	C7	7,335,651	A/A	G/G	C7	48,287,690	C/C	A/A	C8	39,999,025	A/A	T/T	C9	48,546,799	A/A	G/G
C1	38,141,146	T/T	C/C	C2	36,872,331	A/A	G/G	C3	20,868,550	A/A	C/C	C4	3,755,207	G/G	A/A	C5	2,330,507	C/C	T/T	C5	44,248,700	T/T	G/G	C7	7,335,766	C/C	T/T	C7	48,313,751	T/T	C/C	C8	40,004,725	T/T	C/C	C9	48,551,801	A/A	T/T
C1	38,306,910	C/C	T/T	C2	36,872,401	T/T	C/C	C3	20,904,015	A/A	T/T	C4	3,771,547	C/C	G/G	C5	2,372,847	A/A	T/T	C5	44,248,743	T/T	A/A	C7	7,335,802	G/G	T/T	C7	48,313,863	G/G	A/A	C8	40,234,741	T/T	C/C	C9	48,551,868	C/C	A/A
C1	38,412,466	C/C	A/A	C2	36,891,488	G/G	C/C	C3	20,904,046	A/A	T/T	C4	3,881,195	G/G	A/A	C5	2,372,883	T/T	C/C	C5	44,248,746	T/T	A/A	C7	7,454,825	A/A	T/T	C7	48,316,610	G/G	A/A	C8	40,234,744	T/T	C/C	C9	48,551,871	T/T	C/C
C1	38,673,644	A/A	G/G	C2	36,891,502	G/G	A/A	C3	20,904,047	A/A	C/C	C4	3,881,348	A/A	T/T	C5	2,451,539	G/G	A/A	C5	44,248,923	T/T	G/G	C7	7,454,895	G/G	C/C	C7	48,352,241	C/C	T/T	C8	40,236,102	T/T	C/C	C9	48,595,688	G/G	T/T
C1	38,679,259	C/C	T/T	C2	36,891,510	C/C	T/T	C3	20,906,623	G/G	A/A	C4	3,881,371	C/C	G/G	C5	2,451,545	A/A	G/G	C5	44,280,471	C/C	G/G	C7	7,551,315	G/G	A/A	C8	32,318	T/T	A/A	C8	40,236,116	C/C	G/G	C9	48,595,825	T/T	C/C
C1	38,679,648	T/T	C/C	C2	36,968,788	A/A	G/G	C3	20,933,668	G/G	A/A	C4	3,883,482	A/A	G/G	C5	2,451,557	T/T	C/C	C5	44,344,437	G/G	A/A	C7	7,551,326	C/C	T/T	C8	32,362	A/A	C/C	C8	40,236,117	T/T	C/C	C9	48,760,563	T/T	C/C
C1	38,679,696	T/T	G/G	C2	36,977,756	T/T	C/C	C3	20,994,510	C/C	G/G	C4	4,104,108	T/T	A/A	C5	2,466,590	T/T	C/C	C5	44,373,161	A/A	T/T	C7	8,000,412	C/C	A/A	C8	42,954	A/A	C/C	C8	40,240,329	G/G	T/T	C9	48,760,581	T/T	G/G
C1	38,896,384	A/A	G/G	C2	38,274,044	G/G	A/A	C3	21,058,442	C/C	A/A	C4	4,106,533	G/G	T/T	C5	2,486,596	T/T	C/C	C5	44,514,365	G/G	A/A	C7	9,209,827	C/C	T/T	C8	43,027	G/G	C/C	C8	40,249,746	T/T	G/G	C9	48,782,476	A/A	G/G
C1	38,896,495	C/C	T/T	C2	38,402,146	A/A	G/G	C3	21,146,811	A/A	T/T	C4	4,106,627	G/G	A/A	C5	2,486,617	C/C	A/A	C5	44,581,051	T/T	G/G	C7	9,972,843	A/A	C/C	C8	43,097	A/A	G/G	C8	40,249,758	T/T	G/G	C9	48,820,416	T/T	G/G
C1	39,033,789	G/G	T/T	C2	38,938,179	C/C	T/T	C3	21,226,604	G/G	A/A	C4	4,110,771	A/A	G/G	C5	2,486,640	C/C	T/T	C5	44,595,768	T/T	G/G	C7	9,973,018	C/C	T/T	C8	43,133	G/G	C/C	C8	40,302,350	C/C	A/A	C9	48,857,017	C/C	T/T
C1	39,225,043	T/T	A/A	C2	38,979,864	A/A	T/T	C3	21,226,862	C/C	A/A	C4	4,131,802	A/A	G/G	C5	2,524,715	T/T	A/A	C5	44,726,196	C/C	G/G	C7	9,848,022	G/G	C/C	C8	120,480	C/C	G/G	C8	40,302,362	A/A	G/G	C9	48,868,213	G/G	A/A
C1	39,228,180	C/C	T/T	C2	38,979,900	A/A	G/G	C3	21,234,247	A/A	G/G	C4	4,140,691	C/C	A/A	C5	2,652,767	T/T	C/C	C5	44,830,579	T/T	A/A	C7	9,947,023	C/C	G/G	C8	124,111	G/G	T/T	C8	40,304,550	G/G	A/A	C9	48,909,593	G/G	C/C
C1	39,248,282	G/G	A/A	C2	38,979,912	G/G	A/A	C3	21,234,322	C/C	G/G	C4	4,140,824	C/C	T/T	C5	2,713,712	T/T	G/G	C5	44,928,813	C/C	T/T	C7	9,952,533	G/G	A/A	C8	124,249	A/A	G/G	C8	40,324,333	G/G	C/C	C9	48,909,725	T/T	C/C
C1	39,339,420	T/T	C/C	C2	38,979,916	C/C	A/A	C3	21,259,736	G/G	T/T	C4	4,140,830	C/C	T/T	C5	2,774,919	T/T	A/A	C5	44,928,822	A/A	T/T	C7	9,984,462	A/A	G/G	C8	139,017	C/C	G/G	C8	40,326,135	T/T	A/A	C9	48,952,439	C/C	T/T
C1	39,339,432	T/T	C/C	C2	38,980,356	C/C	A/A	C3	21,311,058	C/C	G/G	C4	4,151,054	C/C	A/A	C5	2,816,910	A/A	C/C	C5	44,963,587	C/C	T/T	C7	10,013,329	C/C	A/A	C8	150,103	C/C	T/T	C8	40,326,186	T/T	C/C	C9	48,989,786	A/A	T/T
C1	39,378,874	T/T	C/C	C2	39,029,985	C/C	T/T	C3	21,428,680	G/G	A/A	C4	4,159,751	C/C	T/T	C5	2,816,011	A/A	C/C	C5	44,963,637	A/A	G/G	C7	10,385,138	A/A	G/G	C8	181,514	G/G	A/A	C8	40,326,187	G/G	T/T	C9	49,123,435	G/G	T/T
C1	39,380,852	T/T	G/G	C2	39,053,513	G/G	A/A	C3	21,437,165	A/A	G/G	C4	4,159,772	C/C	T/T	C5	2,816,046	T/T	C/C	C5	45,097,394	C/C	T/T	C7	10,385,189	G/G	A/A	C8	181,570	T/T	A/A	C8	40,328,271	G/G	A/A	C9	49,123,459	T/T	C/C
C1	39,417,224	T/T	C/C	C2	39,053,539	T/T	C/C	C3	21,451,409	T/T	C/C	C4	4,165,893	G/G	T/T	C5	2,816,126	T/T	A/A	C5	45,097,397	C/C	G/G	C7	10,429,750	T/T	C/C	C8	582,227	T/T	C/C	C8	40,335,542	C/C	A/A	C9	49,123,462	G/G	A/A
C1	39,417,257	T/T	C/C	C2	39,068,596	G/G	T/T	C3	21,532,289	C/C	T/T	C4	4,165,948	A/A	C/C	C5	2,881,301	A/A	C/C	C5	45,342,879	T/T	A/A	C7	10,429,762	G/G	A/A	C8	641,629	A/A	T/T	C8	40,335,558	C/C	T/T	C9	49,130,826	A/A	T/T
C1	39,436,086	C/C	T/T	C2	39,071,550	G/G	A/A	C3	21,664,797	C/C	T/T	C4	4,368,757	C/C	A/A	C5	2,893,159	G/G	A/A	C5	45,353,042	G/G	T/T	C7	10,434,271	T/T	G/G	C8	663,150	T/T	C/C	C8	40,336,531	C/C	T/T	C9	49,130,930	A/A	C/C
C1	39,441,121	T/T	C/C	C2	39,229,239	C/C	T/T	C3	21,664,810	G/G	C/C	C4	4,368,844	G/G	A/A	C5	2,893,174	A/A	G/G	C5	45,495,461	A/A	T/T	C7	10,458,366	G/G	A/A	C8	768,835	T/T	A/A	C8	40,336,648	A/A	G/G	C9	49,130,954	A/A	G/G
C1	39,441,158	A/A	G/G	C2	39,229,251	A/A	G/G	C3	21,664,822	T/T	G/G	C4	4,472,340	T/T	C/C	C5	2,893,178	A/A	C/C	C5	45,495,473	C/C	T/T	C7	10,472,204	C/C	A/A	C8	895,901	C/C	A/A	C8	40,390,599	T/T	G/G	C9	49,130,966	A/A	G/G
C1	39,505,284	C/C	T/T	C2	39,230,423	T/T	G/G	C3	21,681,807	G/G	C/C	C4	4,505,086	G/G	C/C	C5	2,893,292	G/G	A/A	C5	45,495,482	C/C	A/A	C7	10,479,488	C/C	G/G	C8	916,627	G/G	C/C	C8	40,452,074	T/T	G/G	C9	49,135,077	G/G	C/C
C1	39,505,296	G/G	A/A	C2	39,230,601	G/G	A/A	C3	21,704,721	A/A	T/T	C4	4,505,218	C/C	T/T	C5	2,893,312	C/C	T/T	C5	45,495,593	C/C	A/A	C7	10,522,013	T/T	A/A	C8	1,235,590	G/G	A/A	C8	40,452,107	T/T	A/A	C9	49,138,874	C/C	T/T
C1	39,615,777	A/A	T/T	C2	39,273,668	A/A	G/G	C3	21,772,960	T/T	C/C	C4	4,569,830	C/C	G/G	C5	2,925,177	G/G	A/A	C5	45,545,136	A/A	G/G	C7	10,543,131	T/T	A/A	C8	1,246,113	A/A	C/C	C8	40,471,199	G/G	T/T	C9	49,138,877	G/G	A/A
C1	39,681,452	C/C	A/A	C2	39,273,764	G/G	C/C	C3	21,773,035	G/G	C/C	C4	4,598,274	T/T	C/C	C5	2,925,192	C/C	T/T	C5	45,585,702	A/A	T/T	C7	10,560,203	C/C	A/A	C8	1,265,426	C/C	A/A	C8	40,475,902	G/G	A/A	C9	49,138,889	C/C	A/A
C1	39,681,824	A/A	G/G	C2	39,273,803	A/A	C/C	C3	21,884,858	A/A	G/G	C4	4,598,298	G/G	C/C	C5	2,930,649	G/G	T/T	C5	45,687,094	T/T	A/A	C7	10,601,411	G/G	A/A	C8	1,279,468	C/C	A/A	C8	40,494,770	G/G	A/A	C9	49,166,747	T/T	C/C
C1	39,729,210	A/A	G/G	C2	39,275,890	C/C	T/T	C3	21,884,863	C/C	A/A	C4	4,598,310	A/A	G/G	C5	2,930,703	T/T	C/C	C5	45,801,810	A/A	G/G	C7	10,601,447	A/A	C/C	C8	1,279,477	T/T	C/C	C8	40,499,203	G/G	T/T	C9	49,245,582	C/C	T/T
C1	39,775,848	T/T	A/A	C2	39,275,974	A/A	T/T	C3	21,905,895	G/G	C/C	C4	4,599,863	T/T	G/G	C5	2,956,549	C/C	T/T	C5	45,801,869	C/C	G/G	C7	10,605,516	T/T	C/C	C8	1,279,504	G/G	A/A	C8	40,499,204	A/A	T/T	C9	49,261,829	C/C	A/A
C1	39,775																																						

Appendix III Cont.

Table S3: *Cont.* : Differences in Allelic variants at various chromosomes positions in parent lines; the cultivated rapid cycling DHSL150 and C07007.

Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007								
C1	40,057,662	A/A	T/T	C2	39,793,693	G/G	T/T	C3	22,091,859	G/G	A/A	C4	4,686,187	G/G	A/A	C5	3,121,046	C/C	A/A	C6	45,875,667	G/G	T/T	C7	10,822,012	G/G	A/A	C8	1,622,060	T/T	A/A	C9	40,563,212	A/A	C/C	C10	49,393,159	T/T	C/C
C1	40,128,242	C/C	T/T	C2	39,793,754	T/T	C/C	C3	22,104,494	G/G	A/A	C4	4,686,223	T/T	A/A	C5	3,142,404	C/C	A/A	C6	45,876,387	C/C	G/G	C7	10,833,717	A/A	C/C	C8	1,857,001	C/C	G/G	C9	40,597,410	G/G	C/C	C10	49,403,864	G/G	A/A
C1	40,148,225	T/T	A/A	C2	39,861,206	C/C	G/G	C3	22,232,225	G/G	A/A	C4	4,705,008	G/G	A/A	C5	3,142,442	G/G	A/A	C6	45,876,477	T/T	G/G	C7	10,833,730	T/T	G/G	C8	1,890,526	C/C	T/T	C9	40,607,829	G/G	A/A	C10	49,406,161	T/T	C/C
C1	40,148,240	A/A	T/T	C2	39,903,192	G/G	C/C	C3	22,235,859	G/G	A/A	C4	4,705,013	G/G	A/A	C5	3,146,531	G/G	A/A	C6	46,160,161	C/C	T/T	C7	10,833,731	C/C	A/A	C8	2,066,832	G/G	T/T	C9	40,726,097	T/T	A/A	C10	49,458,256	G/G	A/A
C1	40,148,249	A/A	T/T	C2	39,924,125	G/G	T/T	C3	22,235,860	C/C	T/T	C4	4,798,867	A/A	G/G	C5	3,157,070	T/T	G/G	C6	46,325,625	C/C	A/A	C7	10,918,564	C/C	T/T	C8	2,066,862	A/A	T/T	C9	40,726,209	C/C	T/T	C10	49,458,281	T/T	A/A
C1	40,259,216	G/G	C/C	C2	40,025,675	T/T	C/C	C3	22,236,418	A/A	G/G	C4	4,965,360	A/A	T/T	C5	3,181,192	A/A	C/C	C6	46,347,670	C/C	A/A	C7	10,958,100	C/C	G/G	C8	2,085,890	C/C	A/A	C9	40,726,227	A/A	G/G	C10	49,474,336	G/G	A/A
C1	40,259,988	T/T	C/C	C2	40,089,061	A/A	C/C	C3	22,264,862	G/G	C/C	C4	4,965,428	A/A	G/G	C5	3,186,622	C/C	A/A	C6	46,347,673	C/C	T/T	C7	10,958,265	C/C	G/G	C8	2,085,909	T/T	A/A	C9	40,726,293	A/A	C/C	C10	49,474,749	G/G	A/A
C1	40,278,926	T/T	G/G	C2	40,089,085	T/T	C/C	C3	22,273,601	G/G	A/A	C4	5,027,007	C/C	G/G	C5	3,186,637	A/A	T/T	C6	46,476,747	A/A	G/G	C7	11,508,072	G/G	A/A	C8	2,100,087	A/A	T/T	C9	40,733,163	G/G	T/T	C10	49,474,824	A/A	G/G
C1	40,345,565	A/A	G/G	C2	40,558,818	G/G	C/C	C3	22,281,369	A/A	G/G	C4	5,108,928	T/T	A/A	C5	3,275,253	C/C	A/A	C6	46,566,330	G/G	C/C	C7	11,509,127	T/T	A/A	C8	2,100,109	C/C	T/T	C9	40,780,307	G/G	A/A	C10	49,474,857	G/G	A/A
C1	40,542,660	C/C	G/G	C2	40,594,505	T/T	G/G	C3	22,293,845	G/G	C/C	C4	5,111,009	G/G	T/T	C5	3,275,256	A/A	T/T	C6	46,566,612	C/C	T/T	C7	11,513,293	G/G	T/T	C8	2,113,565	A/A	G/G	C9	40,780,310	T/T	A/A	C10	49,484,618	A/A	T/T
C1	40,547,379	T/T	G/G	C2	40,594,652	G/G	A/A	C3	22,388,823	A/A	T/T	C4	5,111,014	C/C	G/G	C5	3,277,411	G/G	C/C	C6	46,650,393	C/C	T/T	C7	11,513,961	C/C	A/A	C8	2,113,590	T/T	G/G	C9	40,782,341	G/G	C/C	C10	49,484,627	A/A	C/C
C1	40,547,399	A/A	C/C	C2	40,594,664	G/G	A/A	C3	22,479,153	C/C	T/T	C4	5,115,629	G/G	T/T	C5	3,287,229	C/C	T/T	C6	46,653,448	T/T	C/C	C7	11,514,597	C/C	T/T	C8	2,113,614	A/A	T/T	C9	40,875,431	T/T	A/A	C10	49,484,645	A/A	G/G
C1	40,547,426	A/A	G/G	C2	40,662,288	G/G	C/C	C3	22,495,174	T/T	C/C	C4	5,152,574	T/T	C/C	C5	3,318,677	A/A	C/C	C6	46,654,351	G/G	T/T	C7	11,534,944	C/C	T/T	C8	2,710,241	G/G	T/T	C9	41,012,042	G/G	A/A	C10	49,491,986	G/G	C/C
C1	40,547,437	T/T	A/A	C2	40,692,659	C/C	T/T	C3	22,495,182	G/G	A/A	C4	5,156,618	T/T	G/G	C5	3,341,833	T/T	C/C	C6	46,654,354	G/G	A/A	C7	11,536,176	C/C	G/G	C8	2,710,261	G/G	C/C	C9	41,062,688	G/G	T/T	C10	49,492,787	T/T	C/C
C1	40,547,462	A/A	G/G	C2	40,692,671	G/G	A/A	C3	22,495,219	T/T	C/C	C4	5,182,115	C/C	T/T	C5	3,345,384	T/T	G/G	C6	46,654,387	T/T	C/C	C7	12,233,354	A/A	T/T	C8	2,710,296	A/A	C/C	C9	41,175,006	A/A	C/C	C10	49,492,979	A/A	C/C
C1	40,564,070	T/T	C/C	C2	40,843,582	C/C	G/G	C3	22,495,264	T/T	G/G	C4	5,145,043	G/G	C/C	C5	3,426,500	G/G	T/T	C6	46,658,185	T/T	G/G	C7	12,239,433	G/G	T/T	C8	2,856,696	T/T	A/A	C9	41,271,383	A/A	G/G	C10	49,494,962	C/C	G/G
C1	40,564,082	C/C	A/A	C2	40,844,871	G/G	T/T	C3	22,496,140	T/T	C/C	C4	5,585,447	T/T	C/C	C5	3,458,927	A/A	C/C	C6	46,658,191	G/G	T/T	C7	12,239,944	G/G	T/T	C8	2,856,720	G/G	A/A	C9	41,392,765	G/G	A/A	C10	49,495,162	C/C	G/G
C1	40,566,789	T/T	C/C	C2	40,861,942	A/A	G/G	C3	22,496,228	A/A	T/T	C4	5,585,451	T/T	A/A	C5	3,777,019	G/G	A/A	C6	46,704,377	A/A	C/C	C7	12,240,955	C/C	T/T	C8	2,856,740	A/A	G/G	C9	41,396,963	G/G	A/A	C10	49,496,079	T/T	A/A
C1	40,567,239	G/G	A/A	C2	40,862,009	T/T	C/C	C3	22,496,242	C/C	T/T	C4	5,585,484	C/C	T/T	C5	3,777,038	T/T	G/G	C6	46,704,440	C/C	T/T	C7	12,257,822	T/T	G/G	C8	2,853,884	T/T	C/C	C9	41,397,444	A/A	G/G	C10	49,496,101	G/G	A/A
C1	40,722,596	T/T	C/C	C2	40,916,749	G/G	A/A	C3	22,604,100	T/T	A/A	C4	5,648,831	G/G	A/A	C5	3,815,747	G/G	C/C	C6	46,708,142	A/A	G/G	C7	12,257,832	G/G	C/C	C8	2,896,522	C/C	G/G	C9	41,446,514	C/C	T/T	C10	49,550,885	T/T	C/C
C1	40,797,046	A/A	G/G	C2	40,916,764	G/G	A/A	C3	22,633,930	A/A	T/T	C4	5,648,834	A/A	C/C	C5	3,857,898	A/A	G/G	C6	46,708,249	C/C	G/G	C7	12,257,835	C/C	A/A	C8	2,977,991	G/G	C/C	C9	41,448,274	A/A	G/G	C10	49,550,966	G/G	T/T
C1	40,799,759	T/T	A/A	C2	41,328,671	G/G	A/A	C3	22,633,932	G/G	T/T	C4	5,754,329	C/C	T/T	C5	3,857,864	A/A	G/G	C6	46,708,259	T/T	G/G	C7	12,258,392	T/T	C/C	C8	3,021,778	C/C	G/G	C9	41,448,300	G/G	T/T	C10	49,550,987	T/T	C/C
C1	40,824,790	C/C	T/T	C2	41,328,691	T/T	C/C	C3	22,680,269	T/T	C/C	C4	6,129,047	C/C	G/G	C5	3,857,873	C/C	T/T	C6	46,708,321	C/C	T/T	C7	12,283,411	A/A	G/G	C8	3,021,797	T/T	C/C	C9	41,448,333	G/G	C/C	C10	49,552,987	C/C	T/T
C1	40,848,731	T/T	G/G	C2	41,373,678	A/A	G/G	C3	22,681,403	A/A	G/G	C4	6,295,251	A/A	C/C	C5	3,940,825	C/C	A/A	C6	46,712,526	G/G	A/A	C7	12,797,269	T/T	G/G	C8	3,021,877	C/C	A/A	C9	41,448,343	T/T	C/C	C10	49,552,997	G/G	C/C
C1	41,021,383	A/A	G/G	C2	41,374,024	A/A	G/G	C3	22,773,073	G/G	T/T	C4	6,351,117	G/G	A/A	C5	3,941,521	C/C	T/T	C6	46,832,735	A/A	C/C	C7	12,797,327	A/A	G/G	C8	3,021,900	C/C	T/T	C9	41,448,387	C/C	A/A	C10	49,553,195	C/C	G/G
C1	41,035,066	C/C	T/T	C2	41,419,068	A/A	G/G	C3	22,773,085	T/T	C/C	C4	6,571,438	A/A	C/C	C5	3,941,565	A/A	G/G	C6	2,093,844	C/C	G/G	C7	12,847,513	A/A	C/C	C8	3,022,501	A/A	G/G	C9	41,448,393	G/G	A/A	C10	49,514,588	T/T	G/G
C1	41,035,110	C/C	G/G	C2	41,426,901	T/T	C/C	C3	22,773,097	A/A	T/T	C4	6,784,741	T/T	A/A	C5	3,970,368	T/T	C/C	C6	2,093,846	T/T	G/G	C7	12,847,550	T/T	G/G	C8	3,338,360	G/G	A/A	C9	41,448,465	A/A	C/C	C10	49,680,335	T/T	C/C
C1	41,035,178	A/A	T/T	C2	41,478,648	A/A	T/T	C3	22,925,381	C/C	T/T	C4	6,862,705	A/A	G/G	C5	3,977,194	T/T	C/C	C6	2,093,879	G/G	C/C	C7	12,851,590	G/G	A/A	C8	3,373,760	C/C	A/A	C9	41,454,060	A/A	C/C	C10	49,680,426	C/C	A/A
C1	41,072,171	G/G	A/A	C2	41,515,307	G/G	C/C	C3	22,963,955	C/C	G/G	C4	6,862,713	A/A	G/G	C5	4,066,845	T/T	C/C	C6	2,396,335	T/T	A/A	C7	12,851,603	T/T	C/C	C8	3,373,788	C/C	A/A	C9	41,454,095	G/G	A/A	C10	49,704,064	G/G	A/A
C1	41,072,215	G/G	A/A	C2	41,523,727	G/G	C/C	C3	22,963,963	A/A	T/T	C4	7,315,197	T/T	C/C	C5	4,077,656	A/A	C/C	C6	2,396,356	T/T	C/C	C7	12,881,664	G/G	T/T	C8	3,412,352	C/C	T/T	C9	41,454,101	A/A	T/T	C10	49,704,070	T/T	G/G
C1	41,129,237	T/T	G/G	C2	41,625,120	G/G	C/C	C3	22,963,973	G/G	A/A	C4	7,537,600	T/T	A/A	C5	4,292,328	T/T	A/A	C6	2,396,375	G/G	C/C	C7	13,082,539	T/T	C/C	C8	3,442,987	A/A	C/C	C9	41,546,922	A/A	C/C	C10	49,704,084	G/G	A/A
C1	41,214,058	G/G	C/C	C2	41,625,133	T/T	A/A	C3	23,124,006	T/T	C/C	C4	7,537,624	A/A	T/T	C5	4,741,814	G/G	C/C	C6	2,402,667	T/T	C/C	C7	13,020,274	T/T	C/C	C8	3,445,964	A/A	C/C	C9	41,546,990	T/T	A/A	C10	49,726,280	C/C	T/T
C1	41,236,986	C/C	T/T	C2	41,625,198	T/T	G/G	C3	23,356,680	C/C	T/T	C4	8,086,720	T/T	G/G	C5	4,741,901	G/G	C/C	C6	2,402,775	A/A	T/T	C7	13,409,186	T/T	C/C	C8	3,445,976	A/A	G/G	C9	41,652,120	A/A	G/G	C10	49,726,282	G/G	A/A
C1	41,236,995	C/C	T/T	C2	41,854,182	T/T	C/C	C3	23,615,851	A/A	G/G	C4	8,093,416	A/A	T/T	C5	4,741,958	C/C	T/T	C6	2,747,981	A/A	G/G	C7	14,436,971	C/C	T/T	C8	3,545,001	T/T	C/C	C9	126,203	T/T	A/A	C10	49,734,726	G/G	A/A
C1	41,392,495	A/A	G/G	C2	41,854,196	G/G	A/A	C3	23,623,183	C/C	T/T	C4	8,191,127	A/A	G/G	C5	4,837,794	A/A	T/T	C6	3,003,549	C/C	A/A	C7	14,437,089	T/T	C/C	C8	3,555,441	T/T	A/A	C9	126,668	A/A	G/G	C10	49,760,039	T/T	C/C
C1	41,414,604	C/C	T/T	C2	41,854,483	T/T	G/G	C3	23,635,010	G/G	A/A	C4	8,290,203	G/G	A/A	C5	5,186,309	T/T	G/G	C6	3,003,553	G/G	T/T	C7	14,637,668	G/G	A/A	C8	3,555,446	G/G	C/C	C9	128,829	C/C	A/A	C10	49,815,507	C	

Appendix III *Cont.***Table S3: *Cont.* : Differences in Allelic variants at various chromosomes positions in parent lines; the cultivated rapid cycling DHSL150 and C07007.**

Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007	Chr.	Position	DHSL150	C07007								
C1	41,922,195	T/T	C/C	C2	42,209,673	G/G	C/C	C3	24,342,328	T/T	C/C	C4	9,615,981	G/G	C/C	C5	5,848,684	G/G	C/C	C6	4,315,792	A/A	G/G	C7	16,411,975	T/T	C/C	C8	7,165,130	A/A	G/G	C9	1,091,321	T/T	C/C	C9	50,123,766	A/A	T/T
C1	42,248,858	G/G	A/A	C2	42,209,682	A/A	G/G	C3	24,389,962	G/G	A/A	C4	9,616,238	C/C	T/T	C5	5,848,726	C/C	A/A	C6	4,510,691	G/G	C/C	C7	16,891,153	T/T	C/C	C8	7,165,156	C/C	A/A	C9	1,091,330	C/C	A/A	C9	50,123,812	A/A	C/C
C1	42,257,366	G/G	T/T	C2	42,240,568	A/A	T/T	C3	24,389,965	C/C	G/G	C4	9,860,584	A/A	G/G	C5	5,848,735	A/A	G/G	C6	4,534,587	T/T	G/G	C7	16,947,731	G/G	T/T	C8	7,166,209	A/A	G/G	C9	1,093,399	G/G	A/A	C9	50,174,724	C/C	T/T
C1	42,257,377	G/G	A/A	C2	42,240,669	C/C	A/A	C3	24,390,022	C/C	T/T	C4	10,165,462	T/T	G/G	C5	5,874,169	A/A	G/G	C6	4,586,477	T/T	G/G	C7	16,948,292	T/T	C/C	C8	7,167,710	A/A	G/G	C9	1,143,669	G/G	A/A	C9	50,245,915	A/A	C/C
C1	42,419,096	T/T	C/C	C2	42,240,693	G/G	C/C	C3	24,395,040	A/A	C/C	C4	10,290,930	G/G	A/A	C5	5,968,823	C/C	T/T	C6	4,749,751	T/T	A/A	C7	16,948,307	A/A	T/T	C8	7,243,991	G/G	T/T	C9	1,180,325	T/T	G/G	C9	50,245,938	A/A	G/G
C1	42,419,189	G/G	A/A	C2	42,259,196	A/A	G/G	C3	24,447,923	C/C	T/T	C4	10,291,005	G/G	A/A	C5	6,116,738	A/A	T/T	C6	4,749,776	C/C	T/T	C7	17,005,393	T/T	C/C	C8	7,365,223	T/T	A/A	C9	1,183,807	A/A	C/C	C9	50,245,986	G/G	A/A
C1	42,578,236	A/A	G/G	C2	42,259,320	G/G	A/A	C3	24,453,997	A/A	T/T	C4	10,418,767	C/C	T/T	C5	6,187,326	T/T	C/C	C6	4,749,827	C/C	T/T	C7	17,080,941	G/G	A/A	C8	7,465,060	A/A	T/T	C9	1,254,314	A/A	T/T	C9	50,245,998	G/G	C/C
C1	42,604,779	G/G	C/C	C2	42,992,855	C/C	T/T	C3	24,526,675	G/G	T/T	C4	10,418,771	T/T	G/G	C5	6,187,412	T/T	C/C	C6	4,803,424	C/C	T/T	C7	17,083,533	T/T	C/C	C8	7,534,060	G/G	C/C	C9	1,254,377	A/A	G/G	C9	50,359,708	C/C	T/T
C1	42,604,801	G/G	C/C	C2	43,220,491	A/A	G/G	C3	24,526,732	C/C	G/G	C4	10,418,793	A/A	T/T	C5	6,438,122	A/A	C/C	C6	4,942,916	C/C	T/T	C7	17,134,765	A/A	C/C	C8	7,551,521	G/G	A/A	C9	1,254,393	T/T	C/C	C9	50,428,862	G/G	A/A
C1	42,604,921	C/C	T/T	C2	43,220,504	T/T	A/A	C3	24,802,837	C/C	T/T	C4	10,418,867	A/A	G/G	C5	6,535,267	G/G	T/T	C6	4,969,582	C/C	T/T	C7	17,323,069	C/C	T/T	C8	7,551,537	T/T	C/C	C9	1,340,968	T/T	C/C	C9	50,451,120	G/G	A/A
C1	42,605,505	A/A	T/T	C2	43,381,025	C/C	A/A	C3	24,802,890	A/A	G/G	C4	10,419,026	C/C	G/G	C5	6,535,305	T/T	A/A	C6	5,117,845	C/C	T/T	C7	17,396,193	A/A	G/G	C8	7,735,976	C/C	A/A	C9	1,422,093	A/A	G/G	C9	50,451,124	G/G	A/A
C1	42,605,724	G/G	A/A	C2	43,381,031	C/C	T/T	C3	24,831,156	T/T	C/C	C4	10,594,293	C/C	T/T	C5	6,597,601	A/A	T/T	C6	5,398,858	T/T	C/C	C7	17,449,193	A/A	G/G	C8	7,736,117	A/A	T/T	C9	1,422,099	T/T	G/G	C9	50,484,993	G/G	T/T
C1	42,647,083	C/C	G/G	C2	43,381,065	C/C	A/A	C3	24,963,199	T/T	A/A	C4	10,594,426	C/C	T/T	C5	6,721,411	T/T	C/C	C6	5,437,742	T/T	A/A	C7	17,449,247	A/A	G/G	C8	7,736,139	C/C	G/G	C9	1,422,240	T/T	C/C	C9	50,485,010	G/G	A/A
C1	42,728,428	G/G	A/A	C2	43,381,083	A/A	G/G	C3	24,963,277	G/G	A/A	C4	10,594,461	G/G	A/A	C5	6,767,336	G/G	A/A	C6	5,448,269	A/A	T/T	C7	17,482,582	C/C	T/T	C8	7,736,201	T/T	A/A	C9	1,422,248	A/A	G/G	C9	50,485,041	G/G	T/T
C1	42,730,714	T/T	A/A	C2	43,382,407	A/A	G/G	C3	24,972,779	A/A	G/G	C4	10,594,476	A/A	T/T	C5	6,773,900	G/G	A/A	C6	5,448,290	A/A	G/G	C7	17,748,461	C/C	A/A	C8	7,736,519	C/C	T/T	C9	1,446,388	T/T	C/C	C9	50,485,076	G/G	A/A
C1	42,763,380	C/C	T/T	C2	43,382,420	A/A	G/G	C3	24,972,833	A/A	T/T	C4	11,140,464	A/A	G/G	C5	6,781,924	T/T	G/G	C6	5,477,001	A/A	G/G	C7	17,786,879	T/T	A/A	C8	7,736,583	T/T	C/C	C9	1,446,428	A/A	C/C	C9	50,485,106	G/G	A/A
C1	42,763,428	C/C	T/T	C2	43,526,968	T/T	C/C	C3	24,974,435	A/A	G/G	C4	11,140,471	T/T	C/C	C5	6,788,221	T/T	G/G	C6	5,634,498	G/G	A/A	C7	17,964,867	T/T	G/G	C8	7,832,310	T/T	C/C	C9	1,446,452	C/C	G/G	C9	50,500,333	C/C	T/T
C1	42,800,307	A/A	G/G	C2	43,884,927	C/C	G/G	C3	24,984,415	T/T	A/A	C4	11,303,459	G/G	A/A	C5	7,120,126	G/G	A/A	C6	5,753,097	C/C	T/T	C7	17,995,163	A/A	G/G	C8	7,834,213	C/C	A/A	C9	1,536,437	T/T	C/C	C9	50,500,370	T/T	A/A
C1	42,800,384	A/A	T/T	C2	43,932,381	A/A	G/G	C3	24,985,887	G/G	A/A	C4	11,654,591	T/T	C/C	C5	7,143,184	A/A	G/G	C6	5,753,164	G/G	A/A	C7	18,092,424	T/T	A/A	C8	7,834,215	T/T	C/C	C9	1,549,560	T/T	C/C	C9	50,500,381	G/G	C/C
C1	42,832,844	A/A	T/T	C2	44,276,255	C/C	T/T	C3	24,986,045	A/A	T/T	C4	11,770,139	C/C	T/T	C5	7,143,220	A/A	T/T	C6	5,753,186	T/T	C/C	C7	18,108,427	C/C	T/T	C8	7,838,155	A/A	G/G	C9	1,592,789	C/C	A/A	C9	50,529,295	A/A	G/G
C1	42,858,579	G/G	T/T	C2	44,720,126	A/A	C/C	C3	24,986,084	G/G	A/A	C4	11,862,067	A/A	C/C	C5	7,175,463	T/T	C/C	C6	6,025,454	G/G	A/A	C7	18,108,455	C/C	G/G	C8	7,838,175	G/G	A/A	C9	1,599,220	A/A	G/G	C9	50,529,322	C/C	G/G
C1	42,869,955	T/T	C/C	C2	44,727,338	T/T	C/C	C3	25,008,490	G/G	T/T	C4	11,862,088	A/A	G/G	C5	7,175,484	T/T	C/C	C6	6,028,395	T/T	G/G	C7	18,189,882	C/C	T/T	C8	7,848,040	C/C	G/G	C9	1,600,741	A/A	G/G	C9	50,559,815	C/C	A/A
C1	42,869,983	G/G	C/C	C2	44,781,325	C/C	T/T	C3	25,008,502	G/G	A/A	C4	11,862,117	T/T	G/G	C5	7,181,790	T/T	C/C	C6	6,041,366	C/C	A/A	C7	18,323,602	G/G	A/A	C8	8,044,742	A/A	C/C	C9	1,638,634	G/G	A/A	C9	50,593,275	G/G	A/A
C1	42,912,404	A/A	G/G	C2	44,874,029	T/T	C/C	C3	25,010,451	C/C	G/G	C4	11,862,121	C/C	T/T	C5	7,181,885	A/A	T/T	C6	6,194,965	C/C	T/T	C7	18,372,052	C/C	T/T	C8	8,044,765	C/C	G/G	C9	1,638,653	T/T	C/C	C9	50,593,368	T/T	C/C
C1	43,038,968	T/T	G/G	C2	44,884,648	T/T	C/C	C3	25,043,818	G/G	C/C	C4	12,872,875	A/A	G/G	C5	7,197,113	A/A	G/G	C6	6,206,251	G/G	A/A	C7	18,375,849	T/T	A/A	C8	8,279,242	T/T	C/C	C9	1,639,081	G/G	A/A	C9	50,593,377	T/T	C/C
C1	43,041,797	C/C	T/T	C2	44,884,659	T/T	C/C	C3	25,043,824	G/G	C/C	C4	13,110,361	T/T	C/C	C5	7,201,354	G/G	C/C	C6	6,435,269	G/G	A/A	C7	18,375,886	A/A	T/T	C8	9,292,102	G/G	A/A	C9	1,693,283	G/G	A/A	C9	50,627,533	A/A	G/G
C1	43,041,809	T/T	A/A	C2	44,944,924	C/C	T/T	C3	25,043,842	A/A	C/C	C4	13,148,818	G/G	A/A	C5	7,304,746	G/G	C/C	C6	6,658,992	G/G	A/A	C7	18,389,969	G/G	A/A	C8	9,303,321	A/A	G/G	C9	1,868,696	T/T	C/C	C9	50,627,549	C/C	A/A
C1	43,041,822	A/A	C/C	C2	45,007,831	C/C	T/T	C3	25,118,131	A/A	G/G	C4	13,148,829	G/G	C/C	C5	7,382,702	C/C	T/T	C6	6,718,530	G/G	A/A	C7	18,483,795	C/C	T/T	C8	9,772,174	C/C	A/A	C9	1,868,811	T/T	A/A	C9	50,627,911	A/A	C/C
C1	43,052,092	A/A	G/G	C2	45,007,844	T/T	C/C	C3	25,144,152	C/C	T/T	C4	14,604,076	T/T	C/C	C5	7,446,146	T/T	C/C	C6	6,720,103	T/T	A/A	C7	18,483,799	T/T	G/G	C8	9,793,851	C/C	G/G	C9	1,876,793	G/G	C/C	C9	50,627,923	A/A	T/T
C1	43,057,045	C/C	G/G	C2	45,007,862	T/T	C/C	C3	25,210,820	C/C	T/T	C4	14,604,095	G/G	A/A	C5	7,446,769	T/T	C/C	C6	6,726,970	A/A	T/T	C7	18,728,818	A/A	G/G	C8	9,809,843	A/A	T/T	C9	1,879,022	C/C	T/T	C9	50,639,344	A/A	G/G
C1	43,095,016	T/T	A/A	C2	45,007,911	A/A	T/T	C3	25,210,850	C/C	T/T	C4	15,100,431	A/A	C/C	C5	7,446,876	A/A	G/G	C6	6,755,286	G/G	C/C	C7	18,728,953	T/T	C/C	C8	9,809,893	G/G	C/C	C9	1,896,281	A/A	T/T	C9	50,639,353	A/A	G/G
C1	43,095,018	A/A	G/G	C2	45,263,948	T/T	C/C	C3	25,307,890	G/G	A/A	C4	15,100,444	T/T	G/G	C5	7,460,966	G/G	A/A	C6	6,755,524	G/G	A/A	C7	18,816,116	A/A	C/C	C8	9,824,160	G/G	A/A	C9	1,899,949	T/T	G/G	C9	50,780,003	C/C	A/A
C1	43,095,121	G/G	C/C	C2	45,263,975	G/G	A/A	C3	25,352,024	A/A	T/T	C4	15,100,447	A/A	G/G	C5	7,460,972	A/A	C/C	C6	6,892,110	T/T	C/C	C7	18,816,170	C/C	T/T	C8	9,824,219	T/T	C/C	C9	1,915,714	G/G	A/A	C9	50,780,119	C/C	T/T
C1	43,244,746	A/A	G/G	C2	45,263,978	A/A	G/G	C3	25,458,009	A/A	G/G	C4	15,104,025	C/C	T/T	C5	7,495,973	G/G	C/C	C6	6,892,145	T/T	C/C	C7	19,344,240	C/C	T/T	C8	9,831,231	A/A	T/T	C9	1,915,745	T/T	C/C	C9	50,780,284	T/T	G/G
C1	43,244,830	C/C	G/G	C2	45,299,558	C/C	A/A	C3	25,498,048	C/C	G/G	C4	15,108,395	G/G	A/A	C5	7,531,129	T/T	C/C	C6	6,931,401	C/C	T/T	C7	19,344,256	C/C	T/T	C8	9,831,281	G/G	C/C	C9	2,099,411	T/T	C/C	C9	50,780,293	G/G	C/C
C1	43,269,485	A/A	G/G	C2	45,299,600	T/T	C/C	C3	25,557,714	T/T	A/A	C4	15,108,406	G/G	T/T	C5	7,567,695	A/A	T/T	C6	6,990,166	C/C	T/T	C7	19,560,283	G/G	A/A	C8	9,831,330	C/C	T/T	C9	2,099,460	G/G	A/A	C9	50,780,296	G/G	A/A
C1	43,317,987	G/G	T/T</																																				

Appendix IV

Phenotypic traits measured during the Initial Salt Shock Screening in 2015

Table S4: Plant height measured (cm) and comparison between treated and control *B. oleracea* plants

Geno ID	Name	Plant height						
		Mean Cntr	STDV (x)	SEM	Mean Trt	STDV (x)	SEM	T.test
DHLS150	<i>B. ole(DHSL150)</i>	26.967	7.855	3.207	21.300	6.479	2.645	0.0519954
C07007-S1	<i>B. bourgaei-S1</i>	16.983	3.296	1.346	15.317	3.688	1.505	0.10161522
C10025-DH	<i>B. bourgaei-DH</i>	15.067	0.459	0.187	13.417	0.917	0.375	0.00085717
C10027-DH	<i>B. bourgaei-DH</i>	51.017	8.450	3.450	40.700	12.675	5.175	0.00696582
C07060-S1	<i>B. oleracea-S1</i>	6.333	0.838	0.342	5.350	0.950	0.388	0.00559424
C10125-DH	<i>B. oleracea-DH</i>	14.560	1.730	0.774	12.840	1.249	0.559	0.00245136
C10128-DH	<i>B. oleracea-DH</i>	8.780	1.114	0.498	7.700	0.957	0.428	0.06955741
C07094-S1	<i>B. incana-S1</i>	9.040	1.176	0.526	7.840	0.918	0.410	0.01070963
C13012-DH	<i>B. incana-DH</i>	25.617	4.605	1.880	24.100	4.332	1.769	0.24759905
C13013-DH	<i>B. incana-DH</i>	11.400	2.706	1.105	11.067	2.439	0.996	0.40529205
C07019-S1	<i>B. hilarionis-S1</i>	9.700	2.399	1.199	7.175	0.919	0.460	0.05863275
C13001-DH	<i>B. hilarionis-DH</i>	13.950	1.487	0.607	11.817	0.787	0.321	0.00618253
C07069-S1	<i>B. oleracea-S1</i>	14.400	1.194	0.534	12.740	1.308	0.585	0.09556585
C10132-DH	<i>B. oleracea-DH</i>	24.317	2.616	1.068	18.900	3.058	1.248	0.0062644
C10139-DH	<i>B. oleracea-DH</i>	14.433	1.171	0.478	11.000	2.656	1.084	0.00106038
C07079A-S1	<i>B. oleracea-S1</i>	13.050	2.749	1.122	10.917	2.417	0.987	0.00708092
C10121-DH	<i>B. oleracea-DH</i>	30.800	1.553	0.634	25.100	2.982	1.218	0.00915396
Early Big-DH	<i>B. oleracea-DH</i>	14.420	0.847	0.379	13.680	0.545	0.244	0.06080378
To1000DH3	<i>B. oleracea</i>	45.533	4.160	1.698	42.200	6.263	2.557	0.03611189

Table S4: Plant fresh weight measured (g) and comparison between treated and control *B. oleracea* genotypes

Geno ID	Name	Plant fresh weight						
		Mean Cntr	STDV (x)	SEM	Mean Trt	STDV (x)	SEM	T.test
DHLS150	<i>B. ole(DHSL150)</i>	54.537	14.534	5.933	39.013	9.233	3.769	0.0125835
C07007-S1	<i>B. bourgaei-S1</i>	23.457	3.880	1.584	22.233	7.434	3.035	0.36206453
C10025-DH	<i>B. bourgaei-DH</i>	45.010	8.941	3.650	36.406	9.489	3.874	0.04692781
C10027-DH	<i>B. bourgaei-DH</i>	66.360	15.189	6.201	45.568	10.583	4.320	0.02102509
C07060-S1	<i>B. oleracea-S1</i>	34.605	4.529	1.849	30.310	4.851	1.980	0.09083652
C10125-DH	<i>B. oleracea-DH</i>	55.738	8.651	3.869	46.852	13.528	6.050	0.00280186
C10128-DH	<i>B. oleracea-DH</i>	31.638	10.169	4.548	28.962	13.124	5.869	0.33111763
C07094-S1	<i>B. incana-S1</i>	25.738	2.259	1.010	17.840	3.670	1.641	0.00673984
C13012-DH	<i>B. incana-DH</i>	40.176	4.979	2.033	29.844	8.776	3.583	0.01140266
C13013-DH	<i>B. incana-DH</i>	48.322	13.401	5.471	32.893	7.856	3.207	0.01933901
C07019-S1	<i>B. hilarionis-S1</i>	14.523	2.630	1.315	10.730	3.642	1.821	0.08419381
C13001-DH	<i>B. hilarionis-DH</i>	32.020	7.021	2.866	23.758	5.567	2.273	0.00577378
C07069-S1	<i>B. oleracea-S1</i>	40.030	6.138	2.745	30.628	4.448	1.989	0.00477092
C10132-DH	<i>B. oleracea-DH</i>	40.640	6.197	2.530	32.483	10.001	4.083	0.07875728
C10139-DH	<i>B. oleracea-DH</i>	40.595	7.740	3.160	29.222	6.922	2.826	0.05702414
C07079A-S1	<i>B. oleracea-S1</i>	25.590	3.741	1.527	18.258	8.042	3.283	0.00928704
C10121-DH	<i>B. oleracea-DH</i>	57.428	7.393	3.018	48.802	7.719	3.151	0.04591283
Early Big-DH	<i>B. oleracea-DH</i>	16.596	3.122	1.396	17.448	2.091	0.935	0.30499299
To1000DH3	<i>B. oleracea</i>	10.573	2.256	0.921	9.295	2.542	1.038	0.19973874

*NOTE: Blue = control and Red = treated plants

Appendix IV *Cont.*

Table S5: Plant dry weight (g) measured and comparison between treated and control *B. oleracea* genotype

Geno ID	Name	Plant_dry_weight						
		Mean Cntr	STDV (x)	SEM	Mean Trt	STDV (x)	SEM	T.test
DHLS150	<i>B. ole(DHSL150)</i>	4.215	0.994	0.406	2.802	1.001	0.408	0.00245536
C07007-S1	<i>B. bourgaei-S1</i>	1.695	0.559	0.228	1.623	0.889	0.363	0.44162579
C10025-DH	<i>B. bourgaei-DH</i>	4.420	0.807	0.330	3.466	1.145	0.467	0.02763895
C10027-DH	<i>B. bourgaei-DH</i>	6.280	0.614	0.251	4.195	1.762	0.719	0.01014799
C07060-S1	<i>B. oleracea-S1</i>	5.922	0.780	0.318	5.182	1.008	0.411	0.04537451
C10125-DH	<i>B. oleracea-DH</i>	5.274	0.795	0.355	3.252	1.658	0.741	0.01203719
C10128-DH	<i>B. oleracea-DH</i>	4.128	1.577	0.705	4.138	1.287	0.576	0.49534405
C07094-S1	<i>B. incana-S1</i>	4.836	0.910	0.407	3.100	0.878	0.393	0.01177742
C13012-DH	<i>B. incana-DH</i>	3.222	0.872	0.356	2.510	0.407	0.166	0.12334193
C13013-DH	<i>B. incana-DH</i>	4.167	1.242	0.507	3.078	0.995	0.406	0.12945915
C07019-S1	<i>B. hilarionis-S1</i>	1.478	0.567	0.284	1.085	0.544	0.272	0.10351743
C13001-DH	<i>B. hilarionis-DH</i>	2.347	0.626	0.256	1.620	0.570	0.233	0.00378254
C07069-S1	<i>B. oleracea-S1</i>	4.008	0.858	0.384	2.492	1.248	0.558	0.01765931
C10132-DH	<i>B. oleracea-DH</i>	4.097	0.673	0.275	2.278	1.500	0.612	0.00035099
C10139-DH	<i>B. oleracea-DH</i>	2.845	0.531	0.217	1.745	0.779	0.318	0.01190016
C07079A-S1	<i>B. oleracea-S1</i>	2.100	0.508	0.208	1.060	0.881	0.360	0.01360123
C10121-DH	<i>B. oleracea-DH</i>	6.568	1.603	0.654	4.237	2.270	0.927	0.00597154
Early Big-DH	<i>B. oleracea-DH</i>	4.580	0.691	0.309	4.414	0.448	0.200	0.3891392
To1000DH3	<i>B. oleracea</i>	0.662	0.384	0.157	0.457	0.374	0.152	0.10403032

Table S6: Leaf fresh weight (g) and comparison between treated and control *B. oleracea*

Geno ID	Name	Leaf_fresh_weight						
		Mean Cntr	STDV (x)	SEM	Mean Trt	STDV (x)	SEM	T.test
DHLS150	<i>B. ole(DHSL150)</i>	3.650	1.334	0.545	3.398	0.936	0.382	0.26941808
C07007-S1	<i>B. bourgaei-S1</i>	4.572	1.717	0.701	4.697	1.349	0.551	0.36778793
C10025-DH	<i>B. bourgaei-DH</i>	6.170	1.907	0.779	6.674	1.604	0.655	0.31933598
C10027-DH	<i>B. bourgaei-DH</i>	1.845	0.864	0.353	1.852	1.026	0.419	0.48642139
C07060-S1	<i>B. oleracea-S1</i>	6.950	1.525	0.623	6.602	1.365	0.557	0.26958045
C10125-DH	<i>B. oleracea-DH</i>	4.068	1.577	0.705	5.080	2.459	1.100	0.21275077
C10128-DH	<i>B. oleracea-DH</i>	2.244	0.332	0.148	1.838	0.683	0.305	0.1124702
C07094-S1	<i>B. incana-S1</i>	5.552	0.888	0.397	3.486	0.889	0.398	0.0194206
C13012-DH	<i>B. incana-DH</i>	4.000	0.923	0.377	3.942	0.841	0.343	0.44974495
C13013-DH	<i>B. incana-DH</i>	10.077	3.273	1.336	8.167	1.432	0.585	0.10364402
C07019-S1	<i>B. hilarionis-S1</i>	2.165	0.795	0.398	2.288	1.301	0.651	0.43028355
C13001-DH	<i>B. hilarionis-DH</i>	3.990	0.315	0.129	3.502	0.384	0.157	0.03815183
C07069-S1	<i>B. oleracea-S1</i>	8.400	1.246	0.557	7.523	1.202	0.537	0.13277427
C10132-DH	<i>B. oleracea-DH</i>	6.630	1.263	0.516	6.067	1.012	0.413	0.19188423
C10139-DH	<i>B. oleracea-DH</i>	8.645	2.331	0.952	7.278	2.059	0.841	0.21699351
C07079A-S1	<i>B. oleracea-S1</i>	5.854	0.940	0.384	4.170	0.986	0.402	0.03818132
C10121-DH	<i>B. oleracea-DH</i>	2.993	0.740	0.302	4.268	1.793	0.732	0.00656907
Early Big-DH	<i>B. oleracea-DH</i>	3.062	0.681	0.305	3.664	0.455	0.203	0.12408786
To1000DH3	<i>B. oleracea</i>	0.562	0.230	0.094	0.382	0.209	0.085	0.06465437

*NOTE: Blue = control and Red = treated plants

Appendix IV *Cont.*

Table S7: Leaf dry weight (g) and comparison between treated and control *B. oleracea* genotypes

Geno ID	Name	Leaf_dry_weight						
		Mean Cntr	STDV (x)	SEM	Mean Trt	STDV (x)	SEM	T.test
DHLS150	<i>B. ole(DHSL150)</i>	1.276	0.087	0.035	1.362	0.097	0.039	0.03009605
C07007-S1	<i>B. bourgaei-S1</i>	1.168	0.046	0.019	1.183	0.097	0.039	0.37697599
C10025-DH	<i>B. bourgaei-DH</i>	0.866	0.093	0.038	0.778	0.285	0.116	0.28657752
C10027-DH	<i>B. bourgaei-DH</i>	1.448	0.111	0.045	1.440	0.070	0.028	0.45554613
C07060-S1	<i>B. oleracea-S1</i>	1.612	0.575	0.235	1.307	0.266	0.108	0.14157809
C10125-DH	<i>B. oleracea-DH</i>	1.173	0.296	0.132	1.183	0.243	0.108	0.46004515
C10128-DH	<i>B. oleracea-DH</i>	0.394	0.103	0.046	0.422	0.119	0.053	0.24378646
C07094-S1	<i>B. incana-S1</i>	1.324	0.260	0.116	0.676	0.368	0.164	0.00160936
C13012-DH	<i>B. incana-DH</i>	1.077	0.162	0.066	1.202	0.141	0.057	0.07792988
C13013-DH	<i>B. incana-DH</i>	0.687	0.275	0.112	0.748	0.275	0.112	0.38353459
C07019-S1	<i>B. hilarionis-S1</i>	0.468	0.046	0.023	0.350	0.205	0.103	0.0627886
C13001-DH	<i>B. hilarionis-DH</i>	1.252	0.085	0.035	1.260	0.112	0.046	0.40908348
C07069-S1	<i>B. oleracea-S1</i>	0.636	0.277	0.124	0.806	0.165	0.074	0.19078057
C10132-DH	<i>B. oleracea-DH</i>	0.844	0.121	0.049	1.058	0.187	0.076	0.00610348
C10139-DH	<i>B. oleracea-DH</i>	0.910	0.310	0.127	0.983	0.200	0.082	0.30223312
C07079A-S1	<i>B. oleracea-S1</i>	1.046	0.179	0.073	1.212	0.188	0.077	0.06663583
C10121-DH	<i>B. oleracea-DH</i>	1.280	0.101	0.041	1.252	0.074	0.030	0.30914478
Early Big-DH	<i>B. oleracea-DH</i>	0.518	0.517	0.231	0.256	0.100	0.045	0.14653372
To1000DH3	<i>B. oleracea</i>	0.183	0.022	0.009	0.203	0.022	0.009	0.07588056

Table S8: Leaf Area (cm) and comparison between treated and control *B. oleracea* genotypes

Geno ID	Name	Leaf_area							T.test
		Mean Cntr	STDV (x)	SEM	Mean Trt	STDV (x)	SEM		
DHLS150	<i>B. ole(DHSL150)</i>	264.196	48.693	19.879	226.820	35.513	14.498	0.05461702	
C07007-S1	<i>B. bourgaei-S1</i>	246.934	26.176	10.686	239.307	25.521	10.419	0.37196935	
C10025-DH	<i>B. bourgaei-DH</i>	274.790	34.931	14.260	273.579	19.925	8.135	0.47376935	
C10027-DH	<i>B. bourgaei-DH</i>	153.566	15.745	6.428	144.186	18.686	7.628	0.14716924	
C07060-S1	<i>B. oleracea-S1</i>	461.104	59.149	24.148	343.736	101.529	41.449	0.01164702	
C10125-DH	<i>B. oleracea-DH</i>	269.787	67.376	30.131	232.388	45.127	20.182	0.03174925	
C10128-DH	<i>B. oleracea-DH</i>	224.248	24.481	10.948	196.364	25.483	11.396	0.12106283	
C07094-S1	<i>B. incana-S1</i>	479.805	52.168	23.330	361.331	57.688	25.799	0.04081665	
C13012-DH	<i>B. incana-DH</i>	278.521	22.319	9.112	248.660	25.226	10.298	0.08215804	
C13013-DH	<i>B. incana-DH</i>	449.376	160.901	65.687	409.470	112.514	45.934	0.26095699	
C07019-S1	<i>B. hilarionis-S1</i>	244.796	45.067	22.533	199.280	66.759	33.380	0.01777901	
C13001-DH	<i>B. hilarionis-DH</i>	283.432	34.927	14.259	233.138	31.138	12.712	0.0029676	
C07069-S1	<i>B. oleracea-S1</i>	356.072	51.660	23.103	381.962	73.278	32.771	0.16959192	
C10132-DH	<i>B. oleracea-DH</i>	351.481	28.871	11.787	313.610	45.023	18.381	0.10411561	
C10139-DH	<i>B. oleracea-DH</i>	416.991	86.971	35.506	368.784	76.963	31.420	0.21730497	
C07079A-S1	<i>B. oleracea-S1</i>	410.159	80.402	32.824	328.500	87.808	35.847	0.0356247	
C10121-DH	<i>B. oleracea-DH</i>	279.360	20.970	8.561	290.310	39.549	16.146	0.3236079	
Early Big-DH	<i>B. oleracea-DH</i>	267.267	63.880	28.568	293.899	68.179	30.491	0.24425391	
To1000DH3	<i>B. oleracea</i>	124.796	42.410	17.314	84.414	35.220	14.378	0.05005908	

*NOTE: Blue = control and Red = treated plants

Appendix IV *Cont.*

Table S9: Calculated ratio between different phenotypic traits measured in *B. oleracea* genotypes

Geno ID	Name	Ratio (Cntrl -Trt/Trt)					
		Plant_height	Plant_fresh_weight	Plant_dry_weight	Leaf_fresh_weight	Leaf_dry_weight	Leaf_area
DHLS150	<i>B. ole(DHSL150)</i>	0.266	0.398	0.504	0.074	-0.063	0.165
C07007-S1	<i>B. bourgaei-S1</i>	0.109	0.055	0.044	-0.027	-0.013	0.032
C10025-DH	<i>B. bourgaei-DH</i>	0.123	0.236	0.275	-0.076	0.113	0.004
C10027-DH	<i>B. bourgaei-DH</i>	0.253	0.456	0.497	-0.004	0.006	0.065
C07060-S1	<i>B. oleracea-S1</i>	0.184	0.142	0.143	0.053	0.233	0.341
C10125-DH	<i>B. oleracea-DH</i>	0.134	0.190	0.622	-0.199	-0.008	0.161
C10128-DH	<i>B. oleracea-DH</i>	0.140	0.092	-0.002	0.221	-0.066	0.142
C07094-S1	<i>B. incana-S1</i>	0.153	0.443	0.560	0.593	0.959	0.328
C13012-DH	<i>B. incana-DH</i>	0.063	0.346	0.284	0.015	-0.104	0.120
C13013-DH	<i>B. incana-DH</i>	0.030	0.469	0.354	0.234	-0.082	0.097
C07019-S1	<i>B. hilarionis-S1</i>	0.352	0.353	0.362	-0.054	0.336	0.228
C13001-DH	<i>B. hilarionis-DH</i>	0.181	0.348	0.449	0.139	-0.007	0.216
C07069-S1	<i>B. oleracea-S1</i>	0.130	0.307	0.609	0.117	-0.211	-0.068
C10132-DH	<i>B. oleracea-DH</i>	0.287	0.251	0.798	0.093	-0.202	0.121
C10139-DH	<i>B. oleracea-DH</i>	0.312	0.389	0.630	0.188	-0.075	0.131
C07079A-S1	<i>B. oleracea-S1</i>	0.195	0.402	0.981	0.404	-0.137	0.249
C10121-DH	<i>B. oleracea-DH</i>	0.227	0.177	0.550	-0.299	0.023	-0.038
Early Big-DH	<i>B. oleracea-DH</i>	0.054	-0.049	0.038	-0.164	1.023	-0.091
To1000DH3	<i>B. oleracea</i>	0.079	0.138	0.449	0.472	-0.098	0.478

Appendix V

Phenotypic traits measured second Salt Shock Screening in 2017

Table S10a: Plant height measured (cm) and comparison between treated and control *B. oleracea* plants

Species Name	Plant_height				
	Mean Cntr	SEM	Mean Trt	SEM	T.test
<i>B. ole(DHSL150)</i>	27.083	0.747	23.783	1.198	0.09560859
<i>007B. bourgaei-S1</i>	10.200	1.193	10.833	1.764	0.5
<i>025B. bourgaei-DH</i>	10.480	0.177	9.160	0.289	0.00216685
<i>013B. incana-DH</i>	9.080	0.887	8.660	0.749	0.3744382
<i>001B. hilarionis-DH</i>	8.500	0.764	7.083	0.341	0.043279
<i>060B. oleracea-S1</i>	5.267	0.233	5.000	0.289	0.25
<i>128B. oleracea-DH</i>	6.667	0.601	6.333	0.167	0.39758362
<i>079AB. oleracea-S1</i>	13.040	1.201	14.640	1.336	0.267621
<i>0121B. oleracea-DH</i>	52.333	1.116	32.833	2.845	0.00144172

Table S10b: Leaf fresh weight (g) and comparison between treated and control *B. oleracea*

Species Name	Leaf_fresh_weight				
	Mean Cntr	SEM	Mean Trt	SEM	T.test
<i>B. ole(DHSL150)</i>	7.242	0.560	12.148	1.566	0.01506594
<i>007B. bourgaei-S1</i>	8.837	1.392	11.233	1.726	0.22012941
<i>025B. bourgaei-DH</i>	9.326	0.766	10.258	0.763	0.11853072
<i>013B. incana-DH</i>	9.678	1.399	8.880	0.258	0.36679228
<i>001B. hilarionis-DH</i>	9.072	0.502	10.627	1.099	0.16799302
<i>060B. oleracea-S1</i>	5.007	0.811	5.340	0.752	0.4368595
<i>128B. oleracea-DH</i>	6.677	0.303	10.100	1.940	0.22902935
<i>079AB. oleracea-S1</i>	6.030	0.566	7.022	0.348	0.14234466
<i>0121B. oleracea-DH</i>	7.018	0.644	8.663	1.271	0.19608846

*NOTE: Blue = control and Red = treated plants

Appendix V Cont.

Table S10c: Leaf dry weight (g) and comparison between treated and control *B. oleracea* genotypes

Species Name	Mean Cntr	SEM	Leaf_dry_weight		T.test
			Mean Trt	SEM	
<i>B. ole(DHSL150)</i>	0.834	0.054	1.117	0.099	0.00190081
<i>007B. bourgaei-S1</i>	1.237	0.380	1.547	0.600	0.09939091
<i>025B. bourgaei-DH</i>	1.930	0.194	2.098	0.050	0.13507655
<i>013B. incana-DH</i>	1.943	0.267	2.178	0.132	0.08837646
<i>001B. hilarionis-DH</i>	1.693	0.127	2.423	0.238	0.021277
<i>060B. oleracea-S1</i>	0.923	0.265	1.097	0.176	0.31881423
<i>128B. oleracea-DH</i>	0.730	0.037	1.690	0.408	0.09015754
<i>079AB. oleracea-S1</i>	1.255	0.157	1.233	0.022	0.45157461
<i>0121B. oleracea-DH</i>	1.358	0.221	1.378	0.105	0.45946896

Table S10d: Leaf Area (cm) and comparison between treated and control *B. oleracea* genotypes

Species Name	Mean Cntr	SEM	Leaf_area		T.test
			Mean Trt	SEM	
<i>B. ole(DHSL150)</i>	116.012	7.394	104.897	7.561	0.16906489
<i>007B. bourgaei-S1</i>	163.873	13.756	187.100	4.854	0.1989727
<i>025B. bourgaei-DH</i>	94.083	5.431	87.794	4.261	0.25923234
<i>013B. incana-DH</i>	120.846	10.767	96.460	8.103	0.01178374
<i>001B. hilarionis-DH</i>	152.616	10.434	163.996	20.850	0.30105473
<i>060B. oleracea-S1</i>	89.623	14.386	77.643	12.815	0.41843632
<i>128B. oleracea-DH</i>	117.552	18.466	129.899	26.337	0.35516062
<i>079AB. oleracea-S1</i>	151.396	11.193	151.377	16.230	0.43020526
<i>0121B. oleracea-DH</i>	180.464	15.422	154.840	17.274	0.16125183

*NOTE: Blue = control and Red = treated plants

Appendix VI

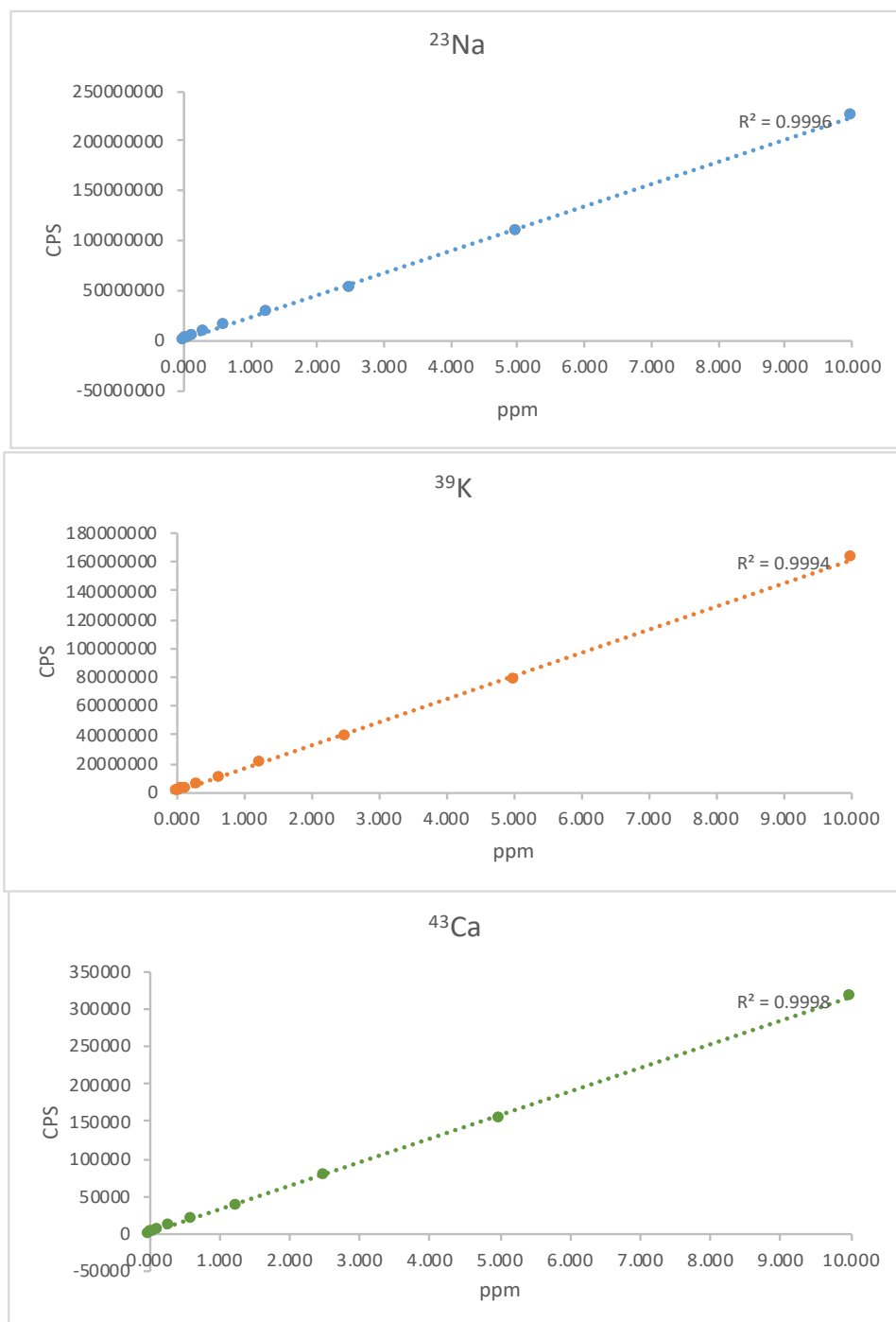


Figure S1: Standard curves established in running samples by using ICP-MS for mineral analysis for sodium (Na), potassium (K) and calcium (Ca) respectively.

Appendix VI Cont.

Different concentration of Na⁺, K⁺ and Ca²⁺ in ppm measured 24 hour post-treatment (24 hpt) (week-six)

Table S11a: Sodium (Na⁺) level (ppm) in 3 replicate each of *B. oleracea* genotypes leaves and comparison between control and treated

Geno ID	Species	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Mean	Mean	STDV	Ct SEM	Ctrl	STDV	Ti SEM	Trt	T.TEST
C10001	<i>B. ole(DHSL150)</i>	0.75	0.30	0.25	2.83	2.57	2.90	0.43	2.77	1.297	0.749	0.174	0.100	0.00258		
C13013	<i>B. incana-DH</i>	1.03	0.82	0.73	2.61	2.11	3.76	0.86	2.83	2.579	1.489	0.843	0.486	0.03328		
C10025	<i>B. bourgaei-DH</i>	0.59	0.68	0.89	3.72	3.05	3.66	0.72	3.48	2.159	1.247	0.371	0.214	0.00309		
C10121	<i>B. oleracea-DH</i>	1.10	0.91	1.17	2.79	3.42	3.79	1.06	3.33	3.177	1.834	0.505	0.292	0.00814		
C13001	<i>B. hilarionis-DH</i>	1.07	0.97	0.93	3.23	2.59	2.30	0.99	2.71	2.963	1.711	0.475	0.274	0.00895		
C07079A	<i>B. oleracea-SI</i>	0.54	0.47	1.62	3.88	4.41	1.44	0.88	3.24	2.628	1.517	1.585	0.915	0.10338		
C07060	<i>B. oleracea-SI</i>	0.68	0.45	0.59	0.98	1.95	3.01	0.57	1.98	1.716	0.991	1.014	0.585	0.07418		
C07007	<i>B. bourgaei-SI</i>	0.47	0.81	0.53	0.55	1.16	2.36	0.60	1.36	1.805	1.042	0.922	0.532	0.14919		
C10128	<i>B. oleracea-DH</i>	0.33	0.10	0.30	0.11	0.94	1.25	0.24	0.77	0.730	0.422	0.588	0.340	0.14685		

Table S11b: Potassium (K⁺) level (ppm) in 3 replicate each of *B. oleracea* genotypes leaves and comparison between control and treated

Geno ID	Species	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Mean	Mean	STDV	Ct SEM	Ctrl	STDV	Ti SEM	Trt	T.TEST
C10001	<i>B. ole (DHSL150)</i>	12.37	6.01	5.43	12.66	9.94	10.76	7.94	11.12	23.815	13.750	1.396	0.806	0.08411		
C13013	<i>B. incana-DH</i>	16.03	10.67	12.81	15.70	15.04	13.69	13.17	14.81	39.510	22.811	1.025	0.592	0.18226		
C10025	<i>B. bourgaei-DH</i>	12.87	8.66	10.55	12.70	12.39	9.47	10.69	11.52	32.078	18.520	1.783	1.029	0.31584		
C10121	<i>B. oleracea-DH</i>	16.77	14.22	17.37	15.41	15.07	17.30	16.12	15.93	48.360	27.921	1.201	0.694	0.39570		
C13001	<i>B. hilarionis-DH</i>	8.46	12.32	13.29	10.28	13.56	13.13	11.36	12.32	34.065	19.667	1.783	1.029	0.12092		
C07079A	<i>B. oleracea-SI</i>	9.30	6.31	11.38	9.53	11.59	9.90	9.00	10.34	26.990	15.583	1.100	0.635	0.28802		
C07060	<i>B. oleracea-SI</i>	15.97	9.51	11.86	16.69	21.32	18.65	12.45	18.89	37.338	21.557	2.324	1.342	0.09118		
C07007	<i>B. bourgaei-SI</i>	3.48	5.59	3.58	2.64	4.27	7.14	4.22	4.68	12.646	7.301	2.277	1.315	0.39619		
C10128	<i>B. oleracea-DH</i>	12.45	1.91	8.33	1.65	9.61	3.98	7.56	5.08	22.687	13.098	4.092	2.362	0.34580		

Table S11c: Calcium (Ca²⁺) level (ppm) in 3 replicate each of *B. oleracea* genotypes leaves and comparison between control and treated

Geno ID	Species	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Mean	Mean	STDV_Ct	SEM_Ctrl	STDV_Tt	SEM_Tr	T.TEST
C10001	<i>B. ole</i> (DHSL150)	2.68	1.16	0.93	2.50	2.13	3.68	1.59	2.77	4.777	2.758	0.809	0.467	0.15062
C13013	<i>B. incana-DH</i>	3.46	2.16	2.80	2.03	3.12	2.77	2.80	2.64	8.414	4.858	0.556	0.321	0.41733
C10025	<i>B. bourgaei-DH</i>	1.89	2.24	2.84	1.73	2.49	2.30	2.33	2.17	6.975	4.027	0.396	0.229	0.28413
C10121	<i>B. oleracea-DH</i>	4.02	3.79	3.72	2.62	2.29	3.53	3.84	2.81	11.531	6.657	0.642	0.371	0.06670
C13001	<i>B. hilarionis-DH</i>	4.60	3.36	2.92	2.40	2.91	3.27	3.62	2.86	10.874	6.278	0.438	0.253	0.20787
C07079A	<i>B. oleracea-Sl</i>	1.97	2.09	6.22	4.29	4.96	6.35	3.43	5.20	10.289	5.940	1.051	0.607	0.08462
C07060	<i>B. oleracea-Sl</i>	2.98	1.40	1.65	1.97	3.55	2.72	2.01	2.75	6.028	3.480	0.792	0.457	0.25562
C07007	<i>B. bourgaei-Sl</i>	2.57	3.07	2.05	2.53	2.60	2.85	2.56	2.66	7.691	4.440	0.168	0.097	0.41194
C10128	<i>B. oleracea-DH</i>	2.10	1.12	1.06	0.29	2.27	0.95	1.43	1.17	4.278	2.470	1.009	0.582	0.39754

Appendix VI Cont.

Different concentration of Na⁺, K⁺ and Ca²⁺ in ppm measured two-weeks post-treatment (2 wkpt)

Table S11d: Sodium (Na⁺) level (ppm) in 3 replicate each of *B. oleracea* genotypes leaves and comparison between control and treated

Geno ID	Species	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	rep 3	Mean	Mean	STDV	Ct	SEM	CtrI	STDV	Ti	SEM	Trt	T.TEST
C10001	<i>B. ole</i> (DHSL150)	1.17	1.47	1.04	6.66	5.24	7.74	1.23	6.55	0.223	0.129	1.253	0.723	0.01231				
C13013	<i>B. incana-DH</i>	0.62	0.30	1.16	1.21	4.18	2.85	0.69	2.75	0.435	0.251	1.491	0.861	0.08426				
C10025	<i>B. bourgaei-DH</i>	0.41	0.91	0.49	2.71	3.98	2.11	0.60	2.93	0.271	0.156	0.952	0.550	0.01531				
C10121	<i>B. oleracea-DH</i>	0.70	0.87	0.78	6.57	11.90	9.60	0.78	9.36	0.085	0.049	2.673	1.543	0.01453				
C13001	<i>B. hilarionis-DH</i>	0.91	4.29	3.25	0.73	5.31	6.22	2.82	4.09	1.731	0.999	2.943	1.699	0.15028				
C07079A	<i>B. oleracea-SI</i>	0.52	0.48	1.15	9.02	0.50	0.86	0.72	3.46	0.377	0.217	4.816	2.781	0.22056				
C07060	<i>B. oleracea-SI</i>	0.53	0.66	0.43	0.92	2.82	4.07	0.54	2.60	0.117	0.068	1.588	0.917	0.07950				
C07007	<i>B. bourgaei-SI</i>	1.91	0.67	0.68	7.66	6.67	2.46	1.08	5.60	0.712	0.411	2.758	1.593	0.04023				
C10128	<i>B. oleracea-DH</i>	0.74	0.18	0.81	0.68	4.26	6.56	0.57	3.83	0.345	0.199	2.964	1.711	0.09991				

Table S11e: Potassium (K⁺) level (ppm) in 3 replicate each of *B. oleracea* genotypes leaves and comparison between control and treated

Geno ID	Species	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Mean	Mean	STDV	Ct	SEM	Ctrl	STDV	Ti	SEM	Trt	T.TEST
C10001	<i>B. ole</i> (DHSL150)	6.77	6.69	9.51	1.79	3.08	4.03	7.66	2.97	1.604	0.926	1.124	0.649				0.00694	
C13013	<i>B. incana-DH</i>	6.66	10.50	12.35	5.66	5.69	9.01	9.84	6.79	2.901	1.675	1.925	1.111				0.05517	
C10025	<i>B. bourgaei-DH</i>	4.41	9.78	4.61	8.47	4.80	4.90	6.26	6.06	3.042	1.757	2.092	1.208				0.47204	
C10121	<i>B. oleracea-DH</i>	4.91	8.04	4.05	6.56	7.62	8.21	5.67	7.46	2.099	1.212	0.836	0.483				0.15363	
C13001	<i>B. hilarionis-DH</i>	6.38	6.37	4.81	6.31	5.12	6.67	5.85	6.03	0.907	0.524	0.812	0.469				0.43034	
C07079A	<i>B. oleracea-SI</i>	9.26	6.14	11.30	3.85	5.24	5.52	8.90	4.87	2.599	1.500	0.895	0.516				0.06192	
C07060	<i>B. oleracea-SI</i>	2.59	10.15	8.28	9.98	7.52	11.84	7.01	9.78	3.936	2.273	2.170	1.253				0.22146	
C07007	<i>B. bourgaei-SI</i>	5.32	7.55	7.49	8.51	3.87	6.14	6.79	6.18	1.271	0.734	2.320	1.340				0.39533	
C10128	<i>B. oleracea-DH</i>	7.65	3.08	11.62	10.21	8.79	5.94	7.45	8.31	4.273	2.467	2.173	1.255				0.41154	

Table S11f: Calcium (Ca²⁺) level (ppm) in 3 replicate each of *B. oleracea* genotypes leaves and comparison between control and treated

Geno ID	Species	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Mean	Mean	STDV	Ct SEM	Ctrl	STDV	Tt SEM	Tt	T.TEST
C10001	<i>B. ole</i> (DHSL150)	4.73	3.16	3.46	2.81	3.19	3.27	3.79	3.09	0.605	0.349	1.959	1.131	0.23242		
C13013	<i>B. incana-DH</i>	1.73	1.95	4.13	0.95	3.08	3.21	2.60	2.41	0.590	0.340	1.353	0.781	0.10675		
C10025	<i>B. bourgaei-DH</i>	2.05	2.91	1.88	3.14	2.28	1.59	2.28	2.34	0.846	0.488	0.151	0.087	0.23635		
C10121	<i>B. oleracea-DH</i>	2.36	2.99	1.79	2.84	3.89	2.85	2.38	3.19	0.800	0.462	0.790	0.456	0.05429		
C13001	<i>B. hilarionis-DH</i>	4.20	2.60	1.71	3.47	2.37	2.06	2.84	2.63	0.468	0.270	0.902	0.521	0.41893		
C07079A	<i>B. oleracea-Sl</i>	1.74	2.02	6.30	3.42	3.01	1.81	3.35	2.75	0.976	0.563	1.111	0.641	0.38690		
C07060	<i>B. oleracea-Sl</i>	3.41	1.49	1.60	1.36	1.38	2.30	2.17	1.68	0.838	0.484	0.996	0.575	0.25488		
C07007	<i>B. bourgaei-Sl</i>	4.45	3.57	3.69	1.36	2.82	4.42	3.90	2.87	0.489	0.282	0.478	0.276	0.05985		
C10128	<i>B. oleracea-DH</i>	5.01	0.64	3.33	2.71	4.28	3.63	2.99	3.54	1.488	0.859	1.670	0.964	0.05811		

Appendix VI Cont.

Different concentration of Na⁺, K⁺ and Ca²⁺ in ppm measured week-eight 24 hr post-treatment (8wk24 hrpt)

Table S11g: Sodium (Na⁺) level (ppm) in 3 replicate each of *B. oleracea* genotypes leaves and comparison between control and treated

Geno ID	Species	Rep1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Mean	Mean	STDV_Ct	SEM_Ctrl	STDV_Tt	SEM_Trt	T.TEST
C10001	<i>B. ole</i> (DHSL150)	0.81	0.89	1.252	1.86	7.02	10.70	0.98	6.53	0.236	0.137	0.172	0.099	0.07560
C13013	<i>B. incana-DH</i>	1.93	0.82	0.210	6.85	3.14	2.18	0.99	4.06	0.872	0.504	1.913	1.104	0.04044
C10025	<i>B. bourgaei-DH</i>	5.63	0.65	0.846	0.47	7.54	8.86	2.37	5.62	2.821	1.629	0.659	0.380	0.26069
C10121	<i>B. oleracea-DH</i>	0.88	1.13	1.300	1.65	1.94	10.45	1.10	4.68	0.209	0.121	3.449	1.991	0.16422
C13001	<i>B. hilarionis-DH</i>	13.10	7.03	5.396	2.44	2.53	6.97	8.51	3.98	4.059	2.344	2.700	1.559	0.16421
C07079A	<i>B. oleracea-S1</i>	6.82	0.96	0.370	0.65	0.74	1.11	2.71	0.84	3.565	2.059	1.126	0.650	0.23833
C07060	<i>B. oleracea-S1</i>	0.42	0.58	0.415	0.71	3.32	1.97	0.47	2.00	0.096	0.055	0.942	0.544	0.08131
C07007	<i>B. bourgaei-S1</i>	3.20	0.44	0.540	9.03	8.01	0.58	1.39	5.87	1.563	0.902	1.529	0.883	0.09390
C10128	<i>B. oleracea-DH</i>	4.73	0.25	0.493	4.90	3.38	5.85	1.82	4.71	2.516	1.452	1.214	0.701	0.09712

Table S11h: Potassium (K⁺) level (ppm) in 3 replicate each of *B. oleracea* genotypes leaves and comparison between control and treated

Geno ID	Species	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Mean	Mean	STDV_Ct	SEM_Ctrl	STDV_Tt	SEM_Trt	T.TEST
C10001	<i>B. ole</i> (DHSL150)	5.51	7.57	6.350	8.30	6.52	6.39	6.48	7.07	1.037	0.599	3.669	2.118	0.32763
C13013	<i>B. incana-DH</i>	6.66	7.39	12.080	6.44	10.31	7.46	8.71	8.07	2.941	1.698	5.079	2.933	0.39867
C10025	<i>B. bourgaei-DH</i>	8.86	7.20	9.163	8.40	10.17	8.34	8.41	8.97	1.056	0.610	3.259	1.882	0.34279
C10121	<i>B. oleracea-DH</i>	11.64	7.34	8.828	9.84	6.74	6.38	9.27	7.65	2.186	1.262	3.988	2.303	0.04876
C13001	<i>B. hilarionis-DH</i>	8.30	5.41	5.502	5.16	18.80	9.70	6.40	11.22	1.639	0.947	1.776	1.025	0.20997
C07079A	<i>B. oleracea-S1</i>	7.40	8.12	6.775	9.64	7.99	10.23	7.43	9.29	0.675	0.390	1.300	0.751	0.11006
C07060	<i>B. oleracea-S1</i>	6.15	7.73	7.671	7.11	7.46	6.13	7.18	6.90	0.897	0.518	2.232	1.289	0.36607
C07007	<i>B. bourgaei-S1</i>	7.55	5.72	7.120	5.83	6.84	7.10	6.80	6.59	0.958	0.553	1.454	0.840	0.41245
C10128	<i>B. oleracea-DH</i>	7.60	4.67	5.853	6.46	8.20	7.55	6.04	7.40	1.477	0.852	4.946	2.856	0.21088

Table S11i: Calcium (Ca²⁺) level (ppm) in 3 replicate each of *B. oleracea* genotypes leaves and comparison between control and treated

Geno ID	Species	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Mean	Mean	STDV_Ct	SEM_Ctrl	STDV_Tt	SEM_Trt	T.TEST
C10001	<i>B. ole</i> (DHSL150)	2.45	3.22	2.027	1.99	3.45	5.73	2.57	3.09	0.605	0.349	1.959	1.131	0.23242
C13013	<i>B. incana-DH</i>	2.17	1.99	3.091	4.04	3.35	3.01	2.42	2.41	0.590	0.340	1.353	0.781	0.10675
C10025	<i>B. bourgaei-DH</i>	4.06	2.69	2.518	3.16	6.14	3.31	3.09	2.34	0.846	0.488	0.151	0.087	0.23635
C10121	<i>B. oleracea-DH</i>	4.15	2.55	3.443	2.81	2.28	2.35	3.38	3.19	0.800	0.462	0.790	0.456	0.05429
C13001	<i>B. hilarionis-DH</i>	3.76	2.99	2.919	2.41	4.81	3.10	3.23	2.63	0.468	0.270	0.902	0.521	0.41893
C07079A	<i>B. oleracea-Sl</i>	4.08	2.16	3.409	2.22	3.34	5.21	3.22	2.75	0.976	0.563	1.111	0.641	0.38690
C07060	<i>B. oleracea-Sl</i>	2.48	0.81	1.517	0.85	1.43	0.98	1.60	1.68	0.838	0.484	0.996	0.575	0.25488
C07007	<i>B. bourgaei-Sl</i>	1.86	2.78	2.600	3.50	4.74	2.93	2.41	2.87	0.489	0.282	0.478	0.276	0.05985
C10128	<i>B. oleracea-DH</i>	3.69	0.86	1.469	4.01	2.48	3.26	2.01	3.54	1.488	0.859	1.670	0.964	0.05811

Appendix VI Cont.

Different concentration of Na⁺, K⁺ and Ca²⁺ in ppm measured two-weeks post-treatment (10week)

Table S11j: Sodium (Na⁺) level (ppm) in 3 replicate each of *B. oleracea* genotypes leaves and comparison between control and treated

Geno ID	Species	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Mean	Mean	STDV	Ct SEM	Ctrl	STDV	Ti SEM	Trt	T.TEST
C10001	<i>B. ole</i> (DHSL150)	1.078	0.908	0.956	0.45	7.29	4.62	0.98	4.12	1.015	0.586	2.270	1.311	0.49518		
C13013	<i>B. incana-DH</i>	3.493	0.150	0.113	0.74	1.53	2.15	1.25	1.48	1.321	0.762	1.292	0.746	0.39298		
C10025	<i>B. bourgaei-DH</i>	0.311	1.621	0.249	0.33	1.11	1.96	0.73	1.14	1.841	1.063	0.480	0.277	0.24542		
C10121	<i>B. oleracea-DH</i>	7.112	0.990	0.968	17.90	3.58	8.20	3.02	9.89	1.273	0.735	1.724	0.995	0.39505		
C13001	<i>B. hilarionis-DH</i>	0.950	0.518	2.376	1.26	2.25	2.04	1.28	1.85	0.445	0.257	1.003	0.579	0.20112		
C07079A	<i>B. oleracea-SI</i>	2.509	2.047	2.414	0.36	0.38	0.28	2.32	0.34	1.032	0.596	3.819	2.205	0.01127		
C07060	<i>B. oleracea-SI</i>	0.172	1.911	0.213	0.21	0.49	0.22	0.77	0.31	0.869	0.502	1.043	0.602	0.14441		
C07007	<i>B. bourgaei-SI</i>	3.072	0.321	0.351	0.59	5.95	2.31	1.25	2.95	0.422	0.243	3.458	1.996	0.21571		
C10128	<i>B. oleracea-DH</i>	2.649	0.606	0.535	0.27	7.41	0.27	1.26	2.65	0.412	0.238	0.289	0.167	0.19303		

Table S11k: Potassium (K⁺) level (ppm) in 3 replicate each of *B. oleracea* genotypes leaves and comparison between control and treated

Geno ID	Species	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Mean	Mean	STDV	Ct SEM	Ctrl	STDV	Ti SEM	Trt	T.TEST
C10001	<i>B. ole</i> (DHSL150)	11.13	3.92	2.03	2.77	2.67	2.02	5.69	2.49	5.520	3.187	4.958	2.863	0.11375		
C13013	<i>B. incana-DH</i>	2.35	4.69	6.05	4.42	4.26	4.13	4.36	4.27	5.389	3.112	5.072	2.928	0.04281		
C10025	<i>B. bourgaei-DH</i>	3.06	4.12	2.32	3.77	2.67	3.84	3.17	3.43	5.903	3.408	2.036	1.176	0.21144		
C10121	<i>B. oleracea-DH</i>	2.26	1.62	1.89	47.32	1.75	3.13	1.93	17.40	7.707	4.450	5.668	3.273	0.27376		
C13001	<i>B. hilarionis-DH</i>	2.75	6.07	4.54	3.51	2.42	4.44	4.45	3.46	4.566	2.636	4.068	2.349	0.25046		
C07079A	<i>B. oleracea-SI</i>	5.32	4.18	2.30	2.72	2.96	3.20	3.93	2.96	4.879	2.817	4.070	2.350	0.26880		
C07060	<i>B. oleracea-SI</i>	3.61	4.03	3.95	3.92	3.59	3.66	3.86	3.72	8.997	5.194	5.927	3.422	0.13564		
C07007	<i>B. bourgaei-SI</i>	4.21	5.72	4.12	3.76	5.60	4.05	4.68	4.47	0.903	0.521	2.164	1.249	0.10907		
C10128	<i>B. oleracea-DH</i>	2.10	11.90	4.80	8.31	8.40	8.31	6.27	8.34	4.008	2.314	3.439	1.985	0.20884		

Table S111: Calcium (Ca²⁺) level (ppm) in 3 replicate each of *B. oleracea* genotypes leaves and comparison between control and treated

Geno ID	Species	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Mean	Mean	STDV	Ct SEM	Ctrl STDV	Ti SEM	Trt T.TEST
C10001	<i>B. ole(DHSL150)</i>	2.898	5.770	5.483	3.94	6.87	4.81	4.72	2.72	1.839	1.062	0.776	0.448	0.48828
C13013	<i>B. incana-DH</i>	4.596	1.896	2.271	2.95	2.49	2.72	2.92	3.23	0.573	0.331	1.164	0.672	0.30317
C10025	<i>B. bourgaei-DH</i>	2.619	2.322	2.178	2.24	2.43	2.61	2.37	2.61	0.382	0.220	0.440	0.254	0.19003
C10121	<i>B. oleracea-DH</i>	1.991	2.179	2.179	29.93	1.62	1.51	2.12	2.66	0.228	0.132	0.621	0.359	0.36511
C13001	<i>B. hilarionis-DH</i>	2.577	1.214	1.922	2.94	2.05	3.02	1.90	2.83	0.493	0.285	0.283	0.163	0.10496
C07079A	<i>B. oleracea-SI</i>	1.224	1.967	3.271	2.22	3.07	2.47	2.15	2.26	0.851	0.491	1.466	0.846	0.02643
C07060	<i>B. oleracea-SI</i>	3.027	3.397	1.011	2.15	3.72	1.17	2.48	1.79	1.282	0.740	1.104	0.637	0.25419
C07007	<i>B. bourgaei-SI</i>	3.551	3.159	3.590	0.95	2.90	2.22	3.43	2.98	0.594	0.343	0.796	0.460	0.49618
C10128	<i>B. oleracea-DH</i>	2.358	4.702	1.906	4.95	3.61	4.95	2.99	1.56	1.054	0.608	0.916	0.529	0.07494

Appendix VII

Scripts used in *B. oleracea* genome alignment during re-analysis of transcriptomic data

Uploading the files

```
>/Users/almustaphalawal/Desktop/Brassica_oleracea.v2.1.37.gff3.gz/  
Genome smrmal@warwick.ac.uk
```

```
find /home/srmal/data/ -name "*.fastq.gz" | xargs -n 1 fastqc -t 6  
-o ./
```

Performing quality test multiqc

```
- multiqc
```

Performing trimming

```
>for i in $(ls *1.fastq.gz | cut -c1-17 | sort -u); do trimmomatic  
PE -threads 20 -phred33 -trimlog trimlogFile.txt  
/home/srmal/data/RNASeqData/fastq_1/${i}1.fastq.gz  
/home/srmal/data/RNASeqData/fastq_1/${i}2.fastq.gz  
${i}_forward_paired.fq.gz ${i}_forward_unpaired.fq.gz  
${i}_reverse_paired.fq.gz ${i}_reverse_unpaired.fq.gz  
ILLUMINACLIP:/home/srmal/adapters/TruSeq3-PE.fa:2:30:10 ; done
```

Align to trimmed reads to the genome

```
>tophat Genome/Boleracea.v2.genome.fasta  
data/RNASeqData/fastq_1/WTCHG_200339_001__forward_paired.fq.gz  
data/RNASeqData/fastq_1/WTCHG_200339_001__reverse_paired.fq.gz -r 74  
-i 50 -I 50000 -p 16 --no-mixed --transcriptome-index  
Genome/Boleracea.v2.cds.fasta
```

```
>tophat Genome/Boleracea.v2.genome.fasta  
data/RNASeqData/fastq_1/${i}_forward_paired.fq.gz  
data/RNASeqData/fastq_1/${i}_reverse_paired.fq.gz -r 74 -i 50 -I  
50000 -p 16 --no-mixed --transcriptome-index  
Genome/Boleracea.v2.cds.fasta
```

To get the Tophat out-put

```
>for i in $(ls /home/srmal/data/RNASeqData/fastq_2/  
*_paired.fq.gz | cut -c1-17 | sort -u); do tophat --output-dir  
/home/u1560846/alalign/${i}_tophat.out
```

```
>/home/u1560846/Bogenome/Boleracea.v2.genome  
/home/srmal/data/RNASeqData/fastq_2/${i}_forward_paired.fq.gz  
/home/srmal/data/RNASeqData/fastq_2/${i}_reverse_paired.fq.gz  
-r 74 -i 50 -I 50000 -p 16 --no-mixed --transcriptome-index  
/home/u1560846/Bogenome/Boleracea.v2.cds.fa ; done
```

Appendix VIII

Table S12a: Normalised data analysis by using two housekeeping genes; B-Tubulin (up-to the yellow mark) and TIP41 (up-to light pink mark)

Species ID: DHLS150																			
Gene Name	Mean Ct_Ctrl	Mean Ct_Trt	SD(Cntrl)	SD(Treated)	%CV (Cntrl)	%CV (Trtred)	SEM(Cntrl)	SEM(Treated)	T.TEST	dCt (Cntrl)	dCt (Trt)	Log Fold	ddCt	Remark	dCt-value	dCt-value	Log Fold	ddCt	Remark
CLC	26.250	26.262	0.6173	0.3168	2.352	1.206	0.356	0.183	0.489981	-2.160	-2.788	0.647	-0.628	Down-regulated	-0.312	-0.942	0.646	-0.630	Down-regulated
CAX3	31.142	31.475	0.1553	0.2524	0.499	0.802	0.090	0.146	0.049055	-4.842	-5.317	0.719	-0.475	Down-regulated	-2.617	-3.672	0.481	-1.055	Down-regulated
ECA2	27.033	28.280	0.5307	0.4570	1.963	1.616	0.306	0.264	0.00971	-1.135	-3.273	0.227	-2.138	Down-regulated	-0.550	-2.088	0.344	-1.538	Down-regulated
KT	25.380	25.180	0.1326	0.0458	0.522	0.182	0.077	0.026	0.081574	-0.125	0.055	1.133	0.180	Up-regulated	0.383	1.165	1.719	0.782	Up-regulated
KUP	22.723	22.762	0.0176	0.1845	0.077	0.811	0.010	0.107	0.364949	2.633	-3.190	0.719	-0.477	Down-regulated	4.098	3.455	0.640	-0.643	Down-regulated
KT2	28.873	28.620	0.1227	0.1992	0.425	0.696	0.071	0.115	0.146439	-2.893	-3.190	0.814	-0.297	Down-regulated	-1.610	-1.968	0.780	-0.358	Down-regulated
NHX1	29.992	30.427	0.3347	0.0869	1.146	0.286	0.198	0.050	0.110763	-4.817	-5.125	0.808	-0.308	Down-regulated	-4.068	-3.948	1.087	0.120	Up-regulated
V-type-a1	26.973	26.310	0.1866	0.3984	0.692	1.514	0.108	0.230	0.030192	-0.965	-0.813	1.111	0.152	Up-regulated	0.398	0.227	0.888	-0.172	Down-regulated
V-type-G	34.067	34.385	0.5636	0.2663	1.654	0.774	0.325	0.154	0.15839	-9.030	-8.737	1.225	0.293	Up-regulated	-7.203	-7.240	0.975	-0.037	Down-regulated

Species ID: C13013-DH																			
Gene Name	Mean Ct_Ctrl	Mean Ct_Trt	SD(Cntrl)	SD(Treated)	%CV (Cntrl)	%CV (Trtred)	SEM(Cntrl)	SEM(Treated)	T.TEST	dCt (Cntrl)	dCt (Trt)	Log Fold	ddCt	Remark	dCt-value	dCt-value	Log Fold	ddCt	Remark
CLC	27.583	27.503	0.4983	0.2977	1.806	1.082	0.288	0.172	0.367121	-3.073	-3.073	1.000	0.000	Down-regulated	-0.995	-1.235	0.847	-0.240	Down-regulated
CAX3	30.832	31.468	0.1233	0.2608	0.400	0.829	0.071	0.151	0.010652	-5.032	-6.227	0.437	-1.195	Down-regulated	-3.128	-4.232	0.465	-1.103	Down-regulated
ECA2	27.073	27.863	0.2426	0.1077	0.896	0.387	0.140	0.062	0.006295	-2.112	-3.582	0.361	-1.470	Down-regulated	-1.965	-2.338	0.772	-0.373	Down-regulated
KT	25.368	25.283	0.1075	0.1686	0.424	0.667	0.062	0.097	0.077979	-0.245	-0.633	0.764	-0.388	Down-regulated	-0.012	0.655	1.587	0.667	Up-regulated
KUP	22.712	22.555	0.1109	0.1572	0.488	0.697	0.064	0.091	0.079989	1.807	1.838	1.022	0.032	Up-regulated	3.700	3.238	0.726	-0.462	Down-regulated
KT2	28.032	27.983	0.2718	0.6476	0.970	2.314	0.157	0.374	0.467589	-2.933	-3.273	0.790	-0.340	Down-regulated	-1.457	-2.072	0.653	-0.615	Down-regulated
NHX1	30.227	30.417	0.2240	0.0601	0.741	0.197	0.129	0.035	0.109277	-6.210	-5.938	1.207	0.272	Up-regulated	-3.732	-4.512	0.582	-0.780	Down-regulated
V-type-a1	26.162	26.068	0.4945	0.8777	1.890	3.367	0.285	0.507	0.458461	-1.293	-1.453	0.895	-0.160	Down-regulated	0.635	0.082	0.681	-0.553	Down-regulated
V-type-G	33.182	33.273	0.1939	0.3890	0.584	1.169	0.112	0.225	0.387667	-8.158	-8.067	1.066	0.092	Up-regulated	-7.258	-6.507	1.684	0.752	Up-regulated

Species ID: C13001-DH																			
Gene Name	Mean Ct_Ctrl	Mean Ct_Trt	SD(Cntrl)	SD(Treated)	%CV (Cntrl)	%CV (Trtred)	SEM(Cntrl)	SEM(Treated)	T.TEST	dCt (Cntrl)	dCt (Trt)	Log Fold	ddCt	Remark	dCt-value	dCt-value	Log Fold	ddCt	Remark
CLC	28.250	28.340	0.6386	0.4196	2.260	1.480	0.369	0.242	0.373177	-1.977	-3.318	0.395	-1.342	Down-regulated	-1.315	-1.012	1.234	0.303	Up-regulated
CAX3	31.115	32.020	0.1929	0.3359	0.620	1.049	0.111	0.194	0.007349	-5.822	-6.202	0.768	-0.380	Down-regulated	-3.677	-4.272	0.662	-0.595	Down-regulated
ECA2	26.568	28.082	0.5774	0.4778	2.173	1.701	0.333	0.276	0.006496	-1.293	-3.047	0.297	-1.753	Down-regulated	-0.518	-1.673	0.449	-1.155	Down-regulated
KT	25.452	26.283	0.2151	0.3497	0.845	1.331	0.124	0.202	0.012756	0.177	-1.485	0.316	-1.662	Down-regulated	0.185	-0.203	0.764	-0.388	Down-regulated
KUP	22.625	22.668	0.0624	0.0679	0.276	0.299	0.036	0.039	0.310818	2.070	2.482	1.330	0.412	Up-regulated	4.042	4.033	0.994	-0.008	Down-regulated
KT2	26.653	27.405	0.4646	0.7776	1.743	2.837	0.268	0.449	0.057846	-1.815	-2.247	0.741	-0.432	Down-regulated	-0.330	-1.148	0.567	-0.818	Down-regulated
NHX1	30.912	31.713	0.2552	0.0525	0.825	0.166	0.147	0.030	0.012425	-6.500	-7.608	0.464	-1.108	Down-regulated	-5.355	-6.027	0.628	-0.672	Down-regulated
V-type-a1	25.690	25.965	0.2714	0.5803	1.057	2.235	0.157	0.335	0.238071	-0.898	-0.893	1.003	0.005	Up-regulated	0.742	0.522	0.859	-0.220	Down-regulated
V-type-G	34.052	34.690	0.6307	0.2787	1.852	0.804	0.364	0.161	0.048762	-8.505	-9.048	0.686	-0.543	Down-regulated	-8.128	-7.362	1.701	0.767	Up-regulated

Appendix VIII Cont.

Table S12b: Normalised data analysis by using two housekeeping genes; B-Tubulin (up-to the yellow mark) and TIP41 (up-to light pink mark)

Species ID: C10121-DH																			
Gene Name	Mean Ct_Ctrl	Mean Ct_Trt	SD(Cntrl)	SD(Treated)	%CV (Cntrl)	%CV (Trted)	SEM(Cntrl)	SEM(Treated)	T.TEST	dCt (Cntrl)	dCt (Trt)	Log Fold	ddCt	Remark	dCt -value	dCt -value	Log Fold	ddCt	Remark
CLC	26.977	27.722	0.5032	0.4357	1.865	1.572	0.291	0.252	0.076292	-2.093	-2.687	0.663	-0.593	Down-regulated	-4.185	-4.730	0.685	-0.545	Down-regulated
CAX3	31.567	32.048	0.2084	0.3602	0.660	1.124	0.120	0.208	0.017972	-6.558	-6.720	0.894	-0.162	Down-regulated	-4.200	-4.852	0.637	-0.652	Down-regulated
ECA2	27.187	28.278	0.0592	0.1054	0.218	0.373	0.034	0.061	0.002792	-1.870	-3.497	0.324	-1.627	Down-regulated	-1.398	-1.878	0.717	-0.480	Down-regulated
KT	25.675	26.178	0.2496	0.1366	0.972	0.522	0.144	0.079	0.032492	-1.383	-1.678	0.815	-0.295	Down-regulated	-0.298	-0.460	0.894	-0.162	Down-regulated
KUP	22.648	22.768	0.0831	0.1279	0.367	0.562	0.048	0.074	0.048587	1.970	2.252	1.216	0.282	Up-regulated	3.768	3.680	0.941	-0.088	Down-regulated
KT2	26.177	26.970	0.3182	0.1480	1.215	0.549	0.184	0.085	0.039113	-1.770	-2.168	0.759	-0.398	Down-regulated	-0.380	-0.798	0.748	-0.418	Down-regulated
NHX1	31.772	32.653	0.3919	0.4216	1.234	1.291	0.226	0.243	0.002829	-7.872	-7.687	1.137	0.185	Up-regulated	-5.612	-6.258	0.639	-0.647	Down-regulated
V-type-a1	24.282	25.003	0.0912	0.6729	0.375	2.691	0.053	0.388	0.086107	-0.887	-1.033	0.903	-0.147	Down-regulated	1.125	0.572	0.681	-0.553	Down-regulated
V-type-G	34.070	34.252	0.4703	0.1457	1.380	0.425	0.272	0.084	0.227721	-9.147	-9.137	1.007	0.010	Up-regulated	-8.273	-7.375	1.864	0.898	Up-regulated

Species ID: C07079A-S																			
Gene Name	Mean Ct_Ctrl	Mean Ct_Trt	SD(Cntrl)	SD(Treated)	%CV (Cntrl)	%CV (Trted)	SEM(Cntrl)	SEM(Treated)	T.TEST	dCt (Cntrl)	dCt (Trt)	Log Fold	ddCt	Remark	dCt -value	dCt -value	Log Fold	ddCt	Remark
CLC	27.010	27.320	0.7873	0.6106	2.915	2.235	0.455	0.353	0.361148	-2.658	-2.905	0.843	-0.247	Down-regulated	-0.542	-0.835	0.816	-0.293	Down-regulated
CAX3	32.098	32.430	0.3230	0.2656	1.006	0.819	0.187	0.153	0.200739	-4.088	-4.575	0.714	-0.487	Down-regulated	-6.362	-6.715	0.783	-0.353	Down-regulated
ECA2	26.382	28.293	0.3176	0.3960	1.204	1.400	0.183	0.229	0.020716	-0.578	-3.830	0.105	-3.252	Down-regulated	-0.502	-2.108	0.328	-1.607	Down-regulated
KT	25.527	25.948	0.1776	0.2696	0.696	1.039	0.103	0.156	0.058418	-0.628	-1.247	0.651	-0.618	Down-regulated	-0.068	0.272	1.266	0.340	Up-regulated
KUP	23.957	24.210	0.6386	0.2778	2.666	1.147	0.369	0.160	0.325017	0.273	-0.048	0.800	-0.322	Down-regulated	2.778	2.135	0.640	-0.643	Down-regulated
KT2	27.525	27.885	0.2037	0.4519	0.740	1.620	0.118	0.261	0.219593	-2.657	-2.778	0.919	-0.122	Down-regulated	-0.828	-1.383	0.681	-0.555	Down-regulated
NHX1	33.080	32.812	0.0577	0.0715	0.174	0.218	0.033	0.041	0.02253	-8.493	-8.317	1.130	0.177	Up-regulated	-7.553	-6.652	1.868	0.902	Up-regulated
V-type-a1	24.547	25.922	1.3462	0.3271	5.484	1.262	0.777	0.189	0.107616	0.252	-1.120	0.386	-1.372	Down-regulated	2.047	0.578	0.361	-1.468	Down-regulated
V-type-G	33.112	34.603	0.2034	0.5283	0.614	1.527	0.117	0.305	0.009218	-8.343	-8.948	0.657	-0.605	Down-regulated	-6.397	-6.928	0.692	-0.532	Down-regulated

Species ID: C07060-S1																			
Gene Name	Mean Ct_Ctrl	Mean Ct_Trt	SD(Cntrl)	SD(Treated)	%CV (Cntrl)	%CV (Trted)	SEM(Cntrl)	SEM(Treated)	T.TEST	dCt (Cntrl)	dCt (Trt)	Log Fold	ddCt	Remark	dCt -value	dCt -value	Log Fold	ddCt	Remark
CLC	28.412	29.040	0.6205	0.3027	2.184	1.042	0.358	0.175	0.089885	-2.247	-2.777	0.693	-0.530	Down-regulated	-1.210	-1.707	0.709	-0.497	Down-regulated
CAX3	31.495	31.915	0.1671	0.2407	0.531	0.754	0.096	0.139	0.005048	-2.270	-3.445	0.443	-1.175	Down-regulated	-4.933	-5.582	0.638	-0.648	Down-regulated
ECA2	26.150	28.578	0.0721	0.0900	0.276	0.315	0.042	0.052	0.00013	-0.288	-2.950	0.158	-2.662	Down-regulated	0.283	-1.377	0.316	-1.660	Down-regulated
KT	26.343	26.377	0.1387	0.2187	0.526	0.829	0.080	0.126	0.289741	-0.715	-0.582	1.097	0.133	Up-regulated	0.355	0.328	0.982	-0.027	Down-regulated
KUP	24.685	25.112	0.6999	0.3497	2.836	1.393	0.404	0.202	0.165226	1.942	0.795	0.452	-1.147	Down-regulated	2.703	2.058	0.639	-0.645	Down-regulated
KT2	29.522	29.138	1.2935	0.3155	4.381	1.083	0.747	0.182	0.283656	-3.385	-2.892	1.408	0.493	Up-regulated	-1.765	-2.042	0.825	-0.277	Down-regulated
NHX1	32.972	33.117	0.4917	0.0580	1.491	0.175	0.284	0.033	0.33634	-7.775	-7.232	1.457	0.543	Up-regulated	-5.673	-5.912	0.848	-0.238	Down-regulated
V-type-a1	26.472	26.297	1.2082	0.1666	4.564	0.634	0.698	0.096	0.417111	-0.580	-0.282	1.230	0.298	Up-regulated	1.333	0.585	0.595	-0.748	Down-regulated
V-type-G	30.413	34.368	1.5944	0.4284	5.242	1.246	0.921	0.247	0.014477	-3.258	-7.948	0.039	-4.690	Down-regulated	-2.368	-5.720	0.098	-3.352	Down-regulated

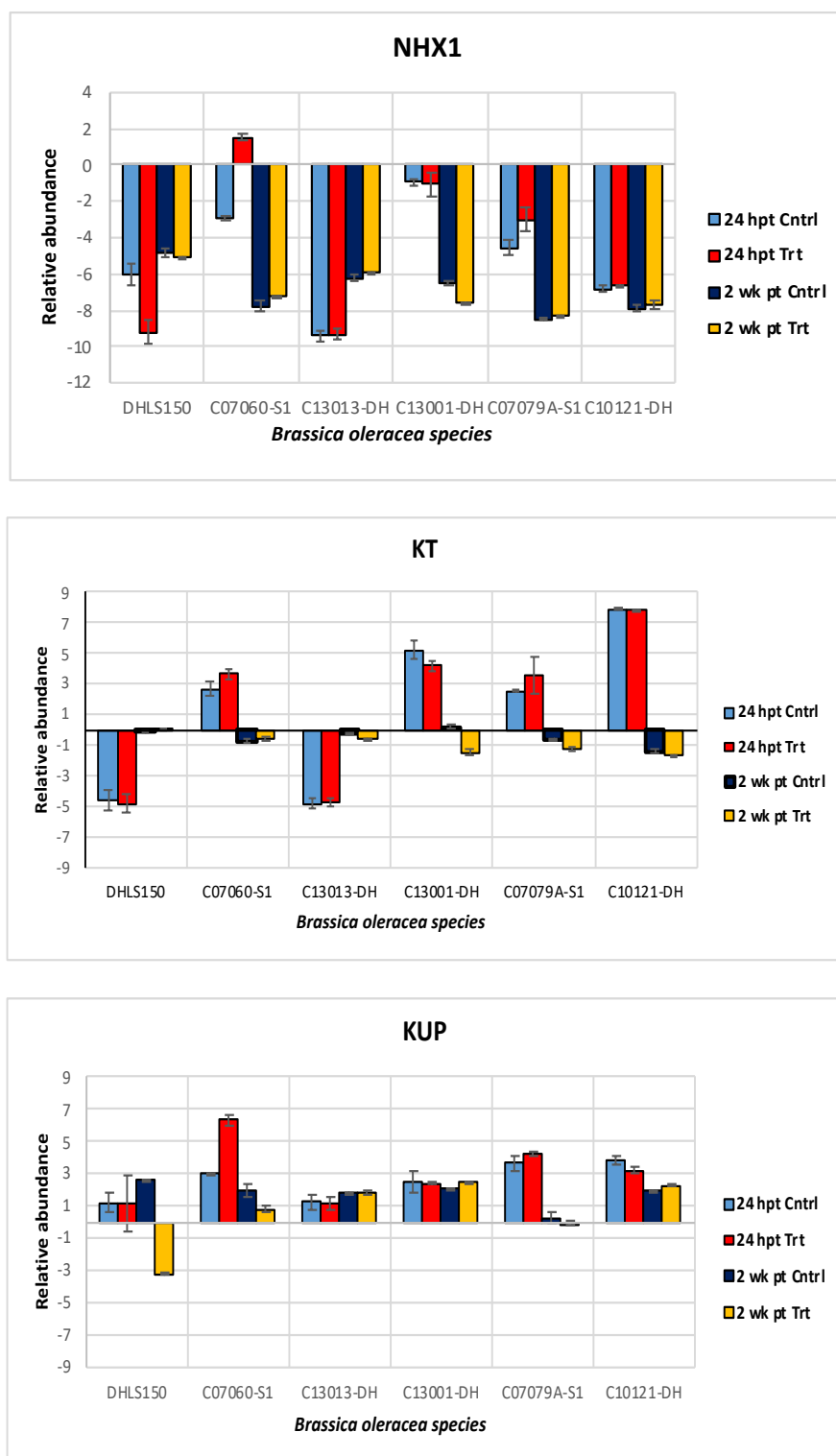
Appendix VIII *Cont.*

Figure S2a: Comparison of relative abundance of Na^+/H^+ exchanger (*NHX1*), potassium transporter (*KT* and *KUP*) between the control and treated normalized by using housekeeping gene B-Tubulin.

Appendix VIII *Cont.*

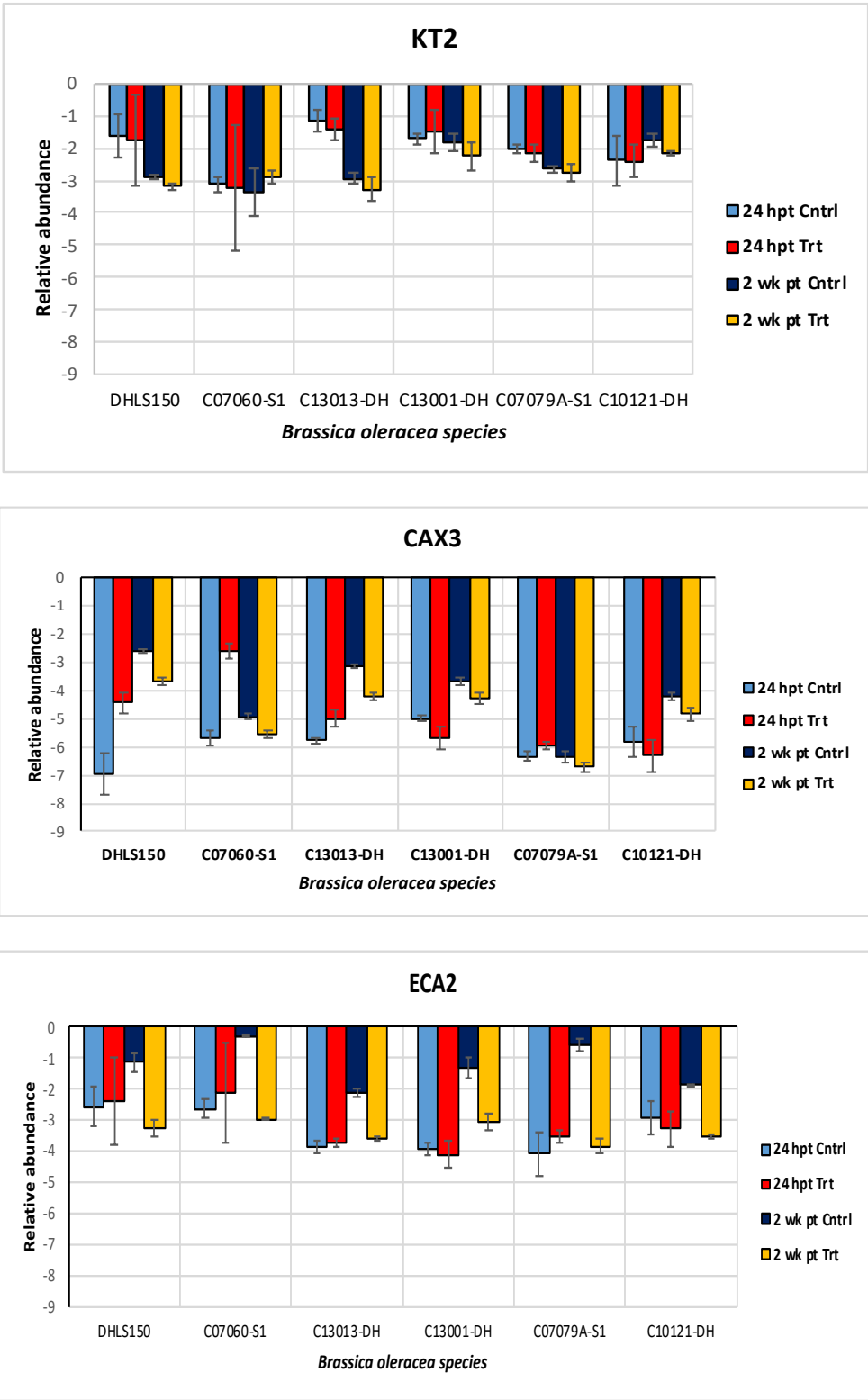


Figure S2b: Comparison of relative abundance of potassium transporter (*KT*), cation exchanger (*CAX3*), and endoplasmic reticulum calcium exchanger (*ECA2*) between the control and treated normalized by using housekeeping gene B-Tubulin.

Appendix VIII *Cont.*

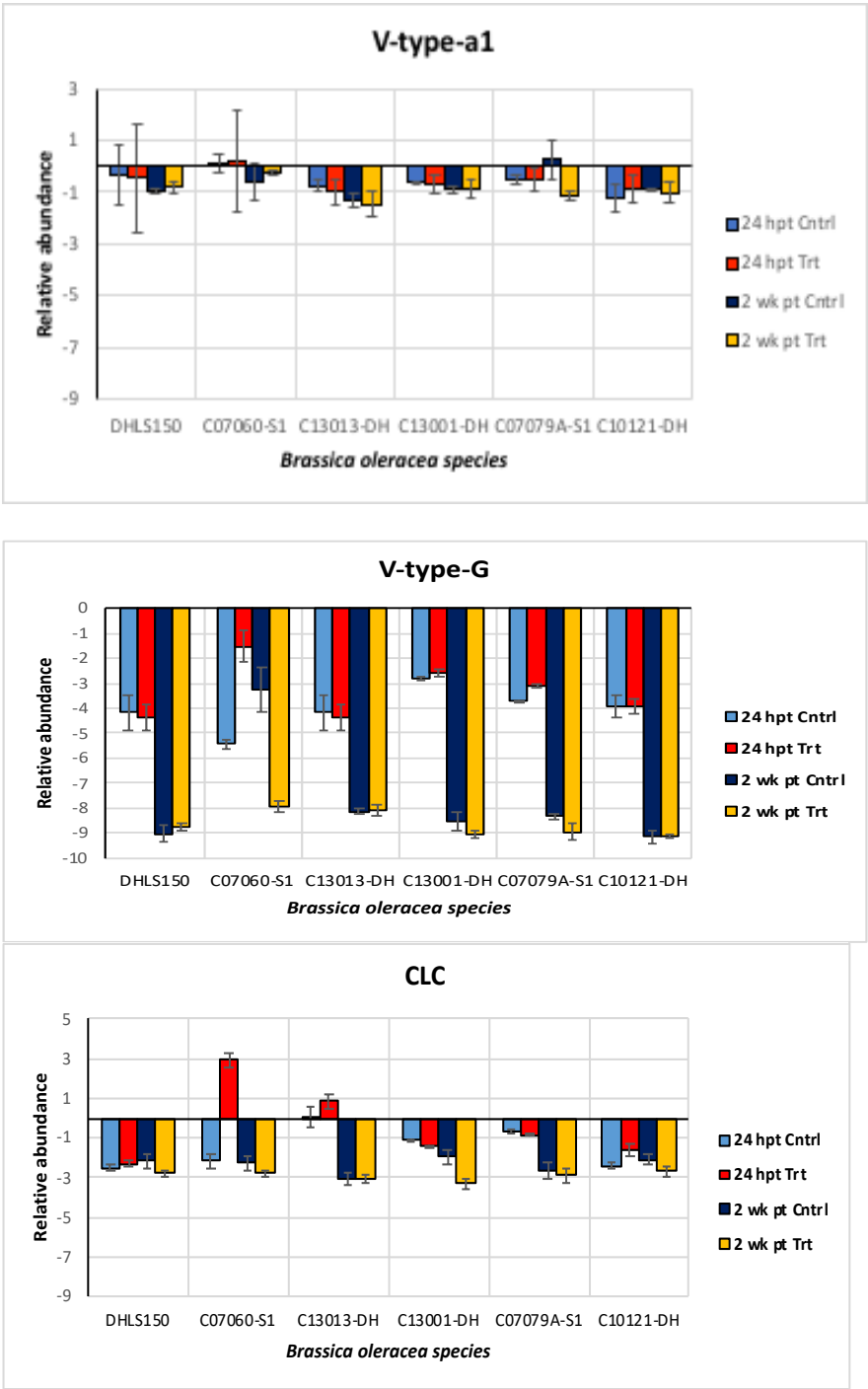


Figure S2c: Comparison of relative abundance of vacuolar proton pumps genes (*V-type a*, and *V-type-G*), and chloride channel B subunit (*CLC-B*), between the control and treated normalized by using housekeeping gene B-Tubulin.

Appendix VIII *Cont.*

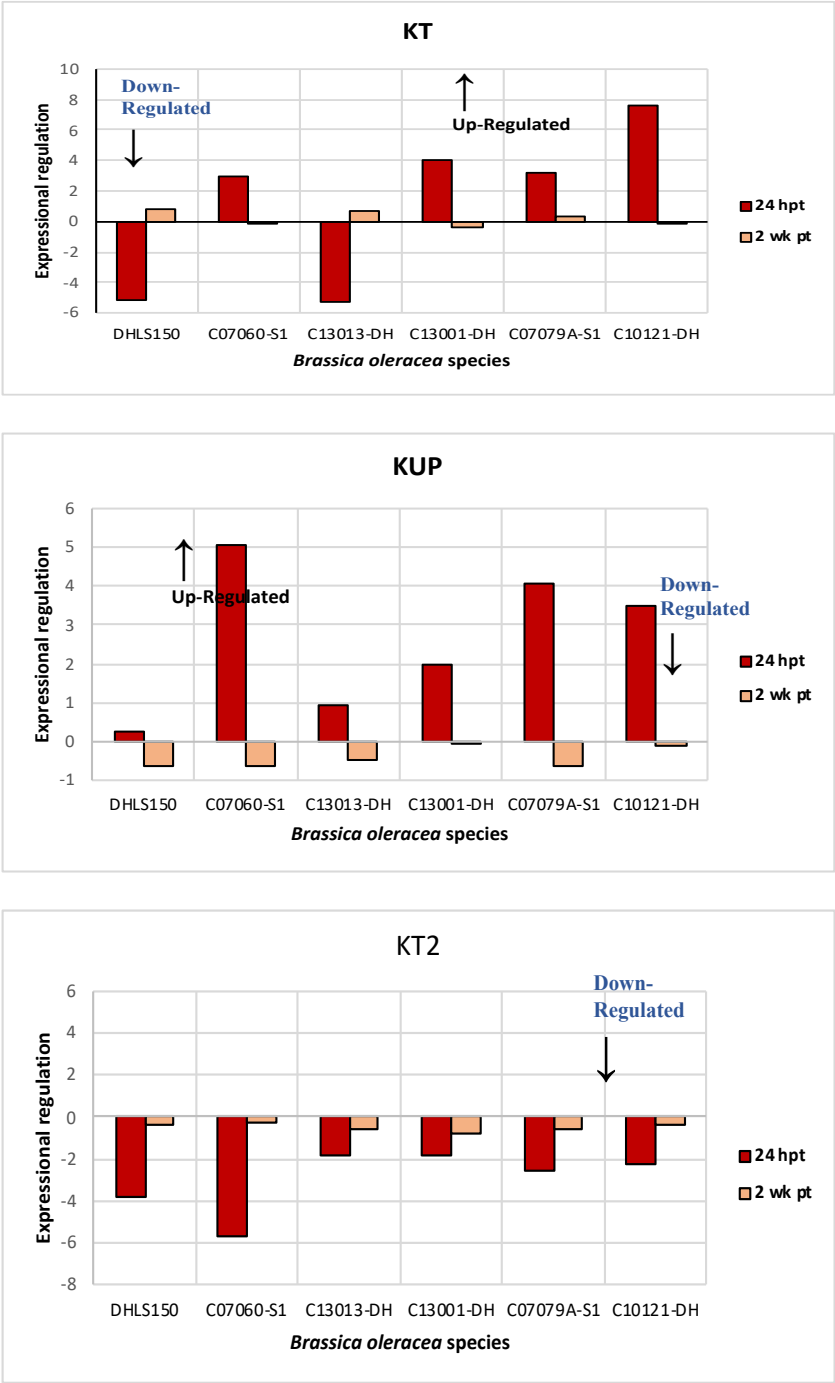


Figure S2d: Comparison of regulation of potassium transporter genes (*KT*, *KUP*), 24 hrpt and 2 weeks (2 wkpt) normalized by using housekeeping gene TIP41.

Appendix VIII *Cont.*

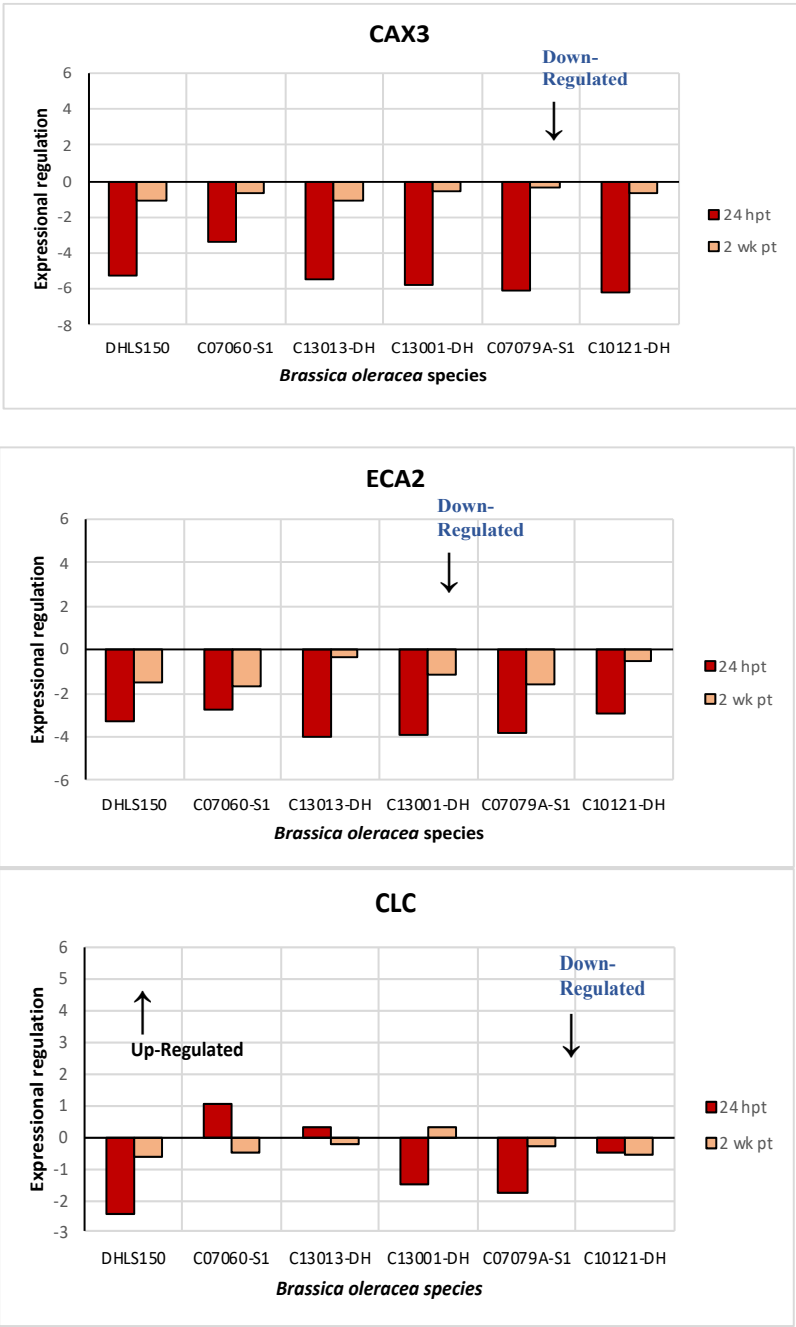


Figure S2e: Comparison of regulation of cation exchanger gene (*CAX3*), and endoplasmic reticulum calcium exchanger (*ECA2*) and chloride channel (*CLC-B*) between 24 hpt and 2 weeks (2 wk) normalized by using housekeeping gene TIP41.

Appendix VIII *Cont.*

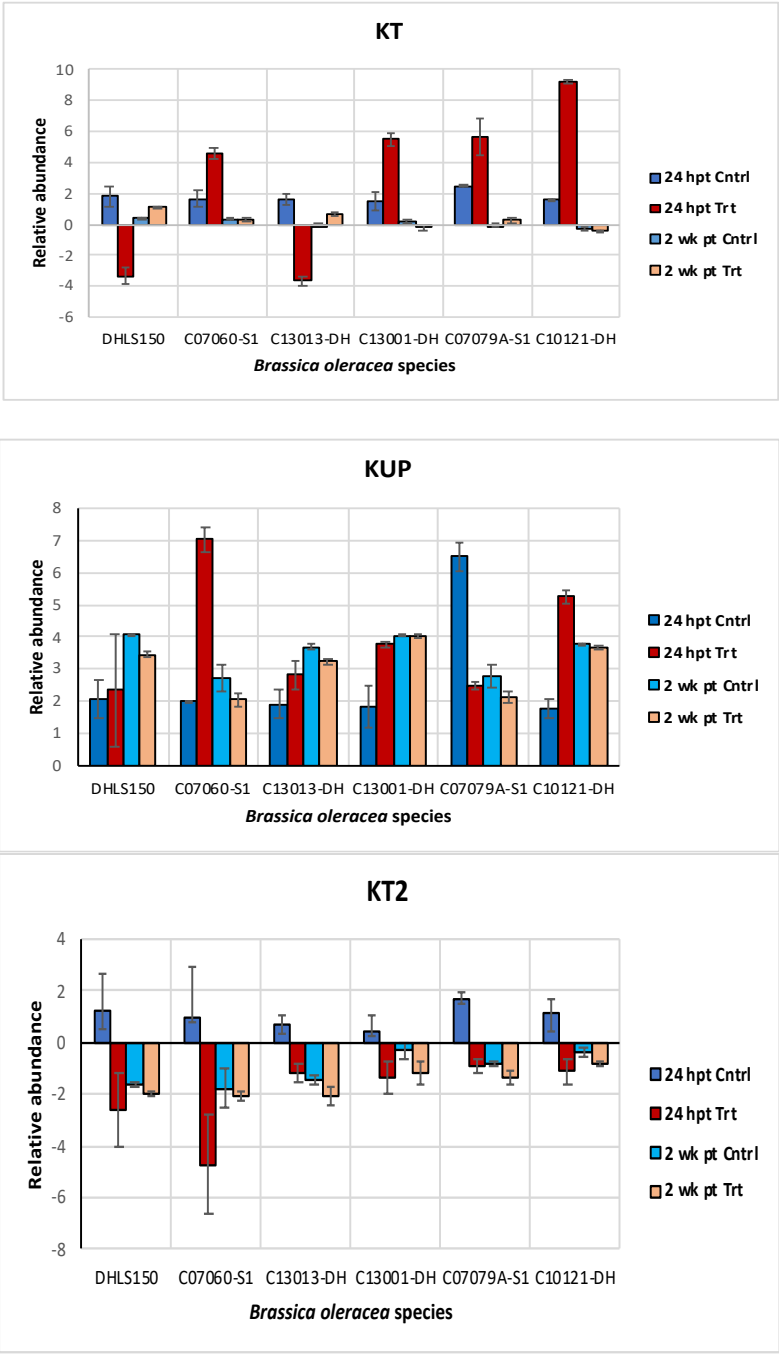


Figure S2f: Comparison of relative abundance of potassium transporter gene (*KT* and *KUP*) between the control and treated normalized by using housekeeping gene TIP41

Appendix VIII *Cont.*

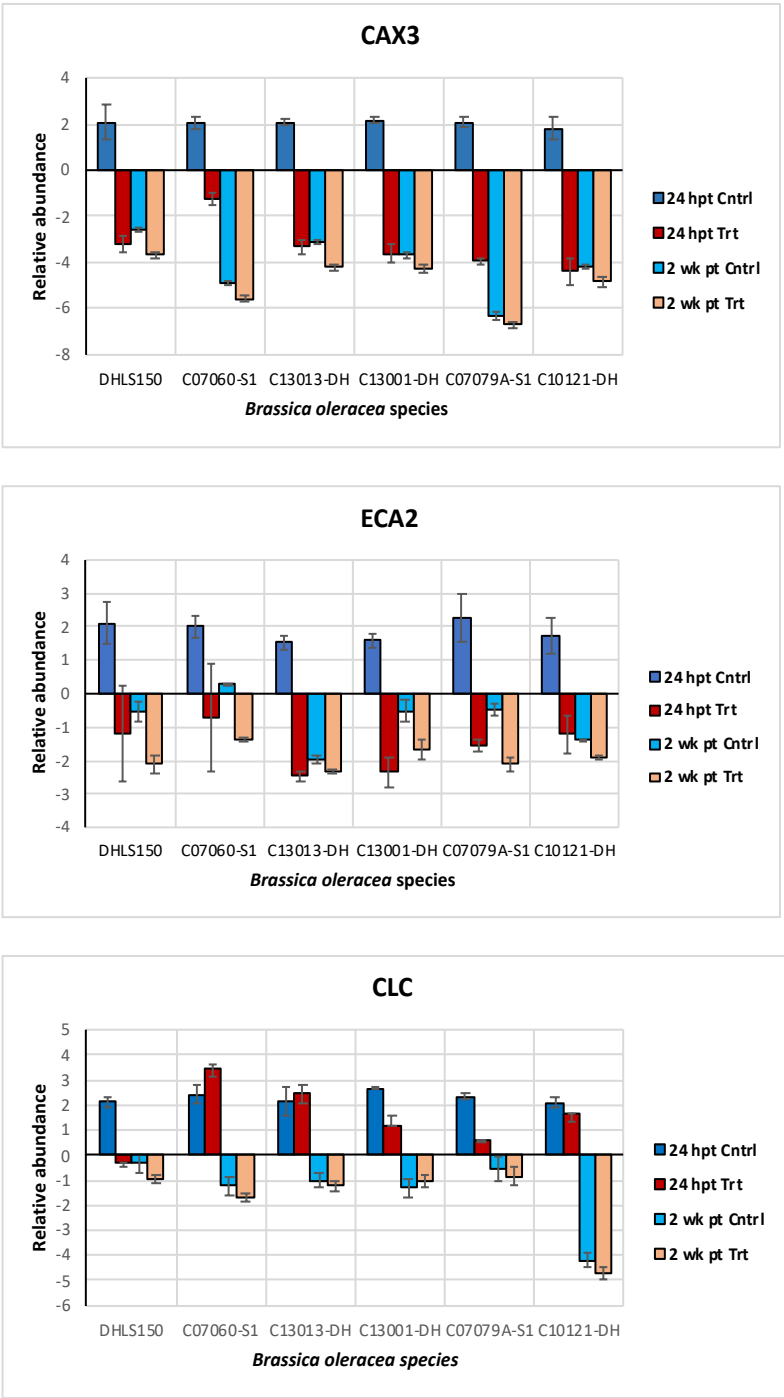


Figure S2g: Comparison of relative abundance of cation exchanger gene (*CAX3*), endoplasmic reticulum calcium transporter gene (*ECA2*) and chloride channel B subunit (*CLC-B*) between the control and treated normalized by using housekeeping gene *TIP41*.

Appendix IX

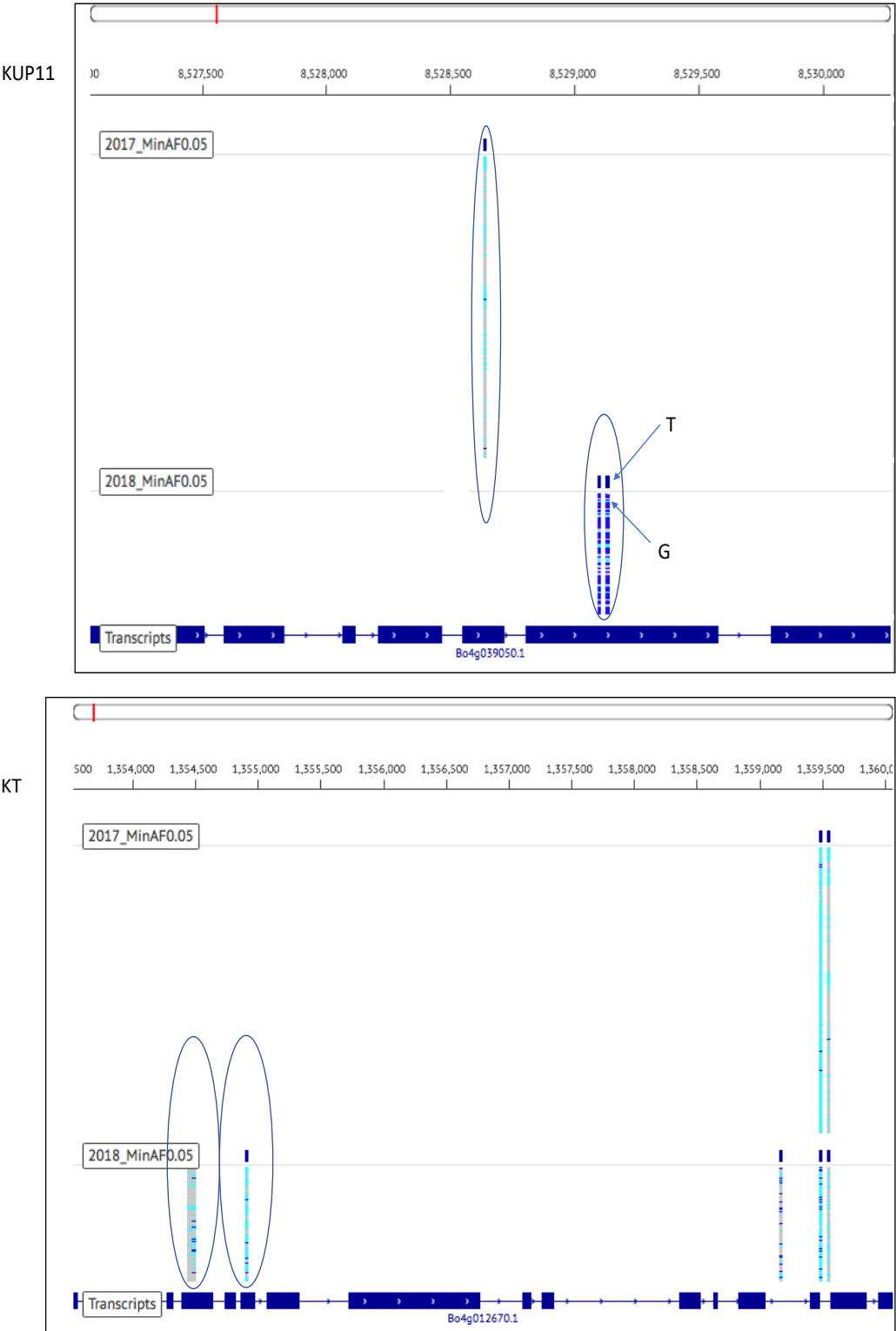


Figure S3a: Images of potassium ion transporter genes indicating heterozygous alleles at exon regions in wildtype S1 *B. oleracea* using IGV.

Appendix IX Cont.

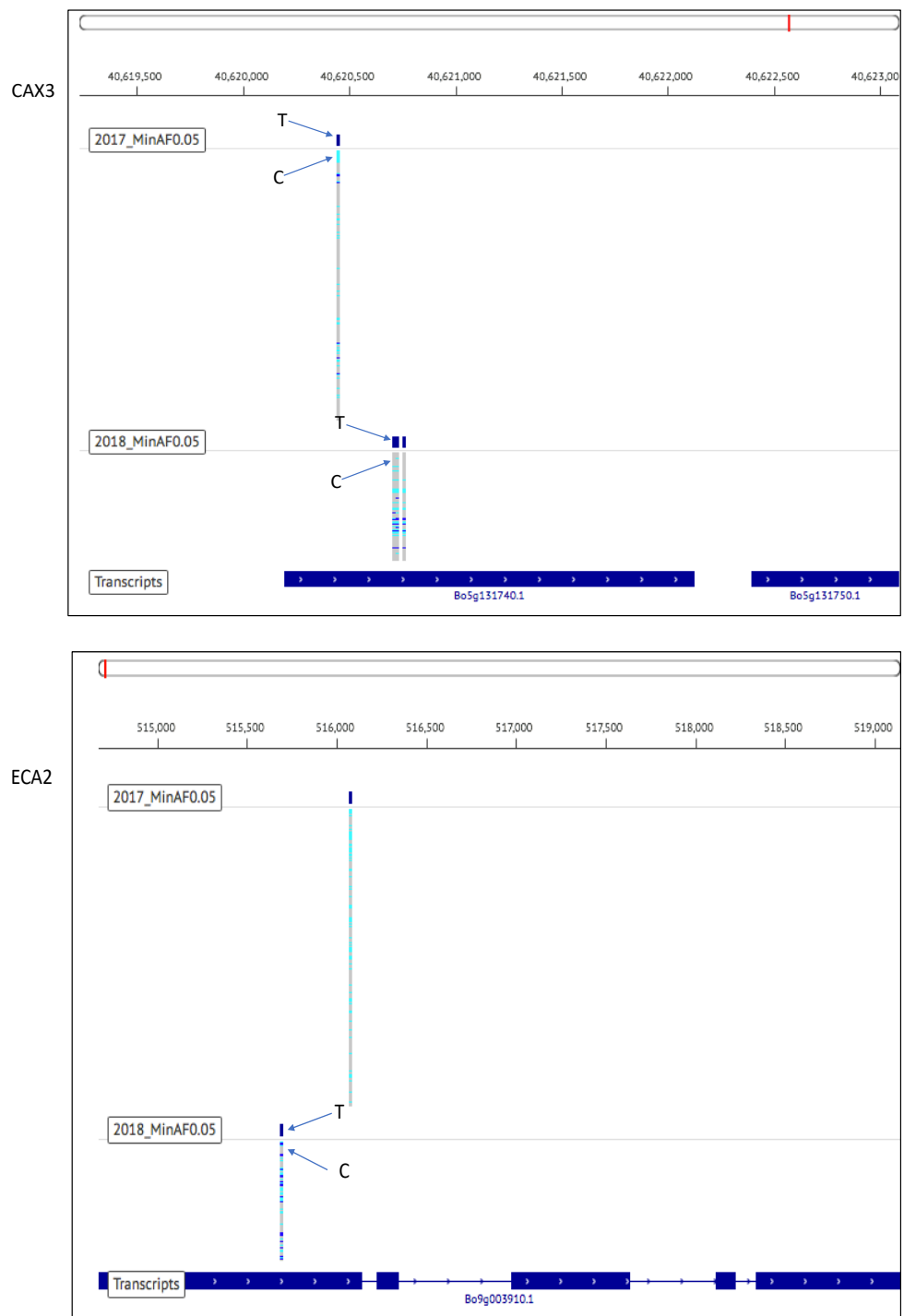


Figure S3b: Images of cation/ H^+ and endoplasmic calcium exchanger showing the presence of heterozygous alleles at exon regions of the transcripts in wildtype S1 *B. oleracea*.

